Chief Scientist & Engineer

Koala Expert Advisory Committee
First milestone report: Review of the Ballina Koala Plan

February 2016

12 February 2016

The Hon Duncan Gay MLC
Minister for Roads and Freight
GPO Box 5341
SYDNEY NSW 2000

Dear Minister

Koala Expert Advisory Committee – Report on the Ballina Koala Plan

On 5 March 2015 you wrote inviting me to chair the Koala Expert Advisory Committee to provide advice on the management of impacts on the koala population resulting from the planned construction of the Woolgoolga to Ballina Pacific Highway Upgrade.

I now submit the first milestone report of the Committee in relation to the Ballina Koala Plan which includes the population viability analysis (PVA) undertaken on behalf of RMS for Section 10 of the upgrade.

The Committee has now reviewed the Ballina Koala Plan and endorses it.

The final PVA presented in the Ballina Koala Plan is the result of a detailed process despite the data limitations and tight timeframes. The Committee has provided advice to RMS and its consultants in the development of the final PVA. This has taken the form of a number of technical workshops including site visits and discussion of mitigation strategies; commissioning genetic analysis and peer comment; and a full day PVA workshop with koala experts from a number of universities. Associate Professor Jonathon Rhodes, from the University of Queensland, and a member of the Committee, has undertaken a review of the Plan and provided comments on the Plan.

The PVA provides estimates of the potential impact of the upgrade on the viability of the koala population as well as the offsetting impacts of habitat restoration and management strategies (including reducing mortality on neighbouring roads or by increasing fecundity through disease management).

This process has improved our knowledge of the Wardell koala population, including providing both data on its demography and genetics. In the case of the genetic analysis, the Wardell population has now been analysed alongside other populations along the NSW coast and the significance the population can now be understood in a larger context.

This process has also demonstrated the limitations of using PVAs to estimate absolute extinction risk. While they are robust in providing relative measures of extinction risk and are useful in comparing the relative outcome of alternative management scenarios. They do not provide an accurate absolute measure of extinction risk.

We suggest that the future use of PVAs in regulatory decisions warrants further consideration at a policy level in the Commonwealth Department of Environment. RMS should offer to share its learnings with the Commonwealth to inform any future application of the PVA technique in regulatory decisions. To this end, it would be useful to further discuss this matter with the Commonwealth at the completion of this process.

The final milestone report will be submitted after review of the Koala Management Plans.

Yours sincerely

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1 INTRODUCTION

The Pacific Highway Upgrade was approved by the NSW Minister for Planning and the Commonwealth Minister for the Environment, subject to strict conditions being met to manage the upgrades impact on the environment.

As part of the conditions of approval, Roads and Maritime Services (RMS) is required to demonstrate whether the impact of the upgrade will be acceptable before construction can begin in the area between Broadwater and Coolgardie (south of Ballina) otherwise known as Section 10. This condition includes conducting a population viability analysis (PVA), for a 50-year timeframe, to demonstrate “the long-term viability of the Ballina koala population, taking into account the impacts resulting from the road upgrade in section 10”.

The conditions further require that PVA outcomes be demonstrated in a Ballina Koala Plan and that the plan be subject to independent expert review and submitted for Commonwealth approval.

The Koala Expert Advisory Committee was convened in February 2015 at the request of the NSW Minister for Roads and Freight to provide advice on the management of impacts on the koala population resulting from the planned construction of the Woolgoolga to Ballina Pacific Highway Upgrade and to review relevant plans and analyses.

The Ballina Koala Plan has now been completed through RMS processes. This Plan, attached at Appendix 3, sets out the results of the population viability analysis (PVA) required by the Commonwealth’s environmental approval.

This is the first milestone report of the Committee which provides an overview of the Committee’s work to date and findings in relation to the review of the Ballina Koala Plan and the PVA.

1.1 PACIFIC HIGHWAY UPGRADE AND CONDITIONS OF APPROVAL

The Roads and Maritime Service (RMS) is upgrading the Pacific Highway from Woolgoolga to Ballina on the north coast of NSW.

RMS received conditional *Environment Protection and Biodiversity Conservation Act 1999* (Cth) (EPBC Act) approval for the upgrade on 14 August 2014 subject to a range of conditions including the progressive preparation and updating of a Koala Management Plan as sections of the road proceed through the construction planning process.

The approval also contains conditions specific to Section 10 – being the section from Broadwater to Coolgardie. This section will impact on the Wardell population of koalas, which has been assessed as being an important population under the EPBC Act.

The conditions specific to Section 10 require that RMS demonstrate that the upgrade of the Pacific Highway will have an acceptable impact on the Wardell Koala population measured over a 50 year timeframe and using a PVA prepared by a qualified expert.

The conditions further require that PVA outcomes be demonstrated in a Ballina Koala Plan and that the plan be subject to independent expert review and submitted for Commonwealth approval.

Once approved by the Commonwealth the approval requires that the Koala Management Plan for the Pacific Highway be updated to incorporate the management measures identified in the Ballina Koala Plan as being important to the ongoing viability of the Wardell Koala population.

Construction can commence once the Koala Management Plan is approved by the Commonwealth.

In implementing the approval conditions the following activities have been undertaken by RMS:

- EcoSure has been commissioned to undertake further koala field survey and demographic analysis from December 2014 to August 2015. This work has been led by Dr Steven Philips of Biolink and includes a genetic analysis conducted by Southern Cross University.
- Dr Rod Kavanagh, Niche Environment and Heritage, have been commissioned to undertake the PVA and prepare the Ballina Koala Plan and Koala Management Plan.
- Associate Professor Jonathan Rhodes, University of Queensland, was commissioned to provide independent advice as required by the approval conditions.

1.2 KOALA EXPERT ADVISORY COMMITTEE (KEAC)

The Koala Expert Advisory Committee (KEAC) was established by NSW Minister for Roads and Freight on 5 March 2015.

KEAC's role, as provided for in its Terms of Reference (Appendix 1), is to provide advice to the Minister for Roads and Freight about:

- The adequacy of the Koala Management Plan prepared for Section 10 in the context of the requirements of the Commonwealth approval conditions
- Recommendations to improve the Koala Management Plan
- Any other advice the Committee considers relevant to improve the management of koalas for future road upgrades

The Terms of Reference require that KEAC report to the Minister on the completion of two milestones, namely:

- review of the population viability analysis undertaken on behalf of RMS for Section 10 of the Woolgoolga to Ballina Pacific Highway upgrade; and
- review of the Koala Management Plans including the Ballina Koala Plan

The Committee is comprised of Professor Mary O'Kane, NSW Chief Scientist and Engineer (Chair), Mr Peter Duncan, Chief Executive, Roads and Maritime Services, and two independent experts, Professor Katherine Belov, the University of Sydney and Associate Professor Jonathan Rhodes, University of Queensland.

Officers from NSW Environment Protection Authority and the NSW Department of Planning and Environment have attended all meetings of the Committee as observers and Commonwealth officers have been invited to each meeting and received the agenda and supporting papers.

To date, the KEAC has met 7 times with its most recent meeting on 17 November 2015.

KEAC has provided advice to RMS and the consultant commissioned to prepare the PVA, to ensure that a detailed process was taken in preparing the PVA and Ballina Koala Plan.

This has included:

- Undertaking a series of technical workshops in Ballina on 9 Feb, 24 April, 12 June and 13 July 2015 with various KEAC members and the experts commissioned by RMS to understand and assist in constructing the PVA parameters.
- Commissioning further genetic analysis by the Australian Museum of the population to inform the PVA model and inviting peer comment by Professor Bill Sherwin of the University of New South Wales and Dr Craig Moritz of the Australian National University on this analysis and the analysis commissioned by RMS and undertaken by Southern Cross University.
- Bringing together 15 koala experts including researchers from the University of Sydney, University of NSW, Queensland University and the University of the Sunshine Coast in a full day workshop in Sydney on 14 October 2015. These researchers were joined by representatives of the Commonwealth Department of Environment, NSW Department of Planning and Environment, NSW Office of Environment and Heritage, Office of the NSW Chief Scientist and Engineer and the NSW Environmental Protection Agency to discuss how the assumptions underpinning PVA parameters can be improved and how sensitivity analysis should be conducted.

2 REVIEW OF THE BALLINA KOALA PLAN

The Ballina Koala Plan is a requirement of the Commonwealth Department of Environment approval conditions (Appendix 2).

The Plan is required to contain:

1. Population viability modelling of the Ballina Koala population over a time period of no less than 50 years, taking into account the impacts resulting from the road upgrade in Section 10. The modelling is expected to consider the proposed route and any proposed avoidance or mitigation measures.
2. A peer review of the modelling by a suitably qualified expert
3. Additional avoidance, mitigation or offsets, beyond those required by the NSW approval conditions, proposed to minimize the impacts to the Ballina Koala population. Including evidence that these measures have been included in the modelling.
4. Discussion of the future viability of the Ballina Koala population

The Plan needs to demonstrate that the impacts to the koala population are acceptable.

The Plan has been prepared for RMS by Dr Rod Kavanagh, Niche Environment and Heritage.

Koala management plans (KMPs) are also required for relevant stages of the upgrade, including stages 5, 9 and 10. The KMPs are required to demonstrate the ongoing survival of the Koala populations at Coolgardie/Bagotville, Broadwater and Woombah/Iluka

The KMPs detail how impacts will be minimised and managed for koala populations and areas of potential koala habitat. They identify the management actions to be carried out to ensure the long-term survival of this species in the area of the project.

The KMPs are required to include details such as: survey results, population status, habitat use and movement patterns, habitat areas likely to be fragmented or isolated, including the results of SPOT assessment and radio tracking, detailed description, location and justification of all proposed avoidance and mitigation measures, revegetation strategy to increase connectivity adjacent to the upgrade and leading to crossing locations, and details of the proposed monitoring methodology to ensure the effectiveness of the mitigation measures and the ongoing survival of the populations.

For Section 10 of the upgrade, the KMP cannot be completed until the Ballina Koala Plan has been approved by the Commonwealth Minister for Environment.

2.1 POPULATION VIABILITY MODELLING

The Ballina Koala Plan is informed by a population viability analysis (PVA). PVA is a method of risk assessment used in conservation biology to estimate the likelihood of a population's extinction and compare proposed management options and assess existing or planned recovery efforts.

Discussion of PVAs and their utility can be found in Section 3 of the Ballina Koala Plan (Appendix 3) and in the review document from Associate Professor Rhodes (Appendix 4).

The Ballina Koala Plan and PVAs have been developed by Niche Environment and Heritage under contract with RMS. The input data was provided by EcoSure (capture and survey program, report in Appendix 1 of the Ballina Koala Plan), with genetic data from the Australian Museum and Southern Cross University which were also under contract to RMS. During the phases of the development of the PVA and Ballina Koala Plan, Niche Environment and Heritage was invited to attend the various workshops and relevant KEAC meetings. More information on the process is at Appendix 3.

The following observations about the role of PVAs have underpinned KEACs approach to developing its advice and provide important context for its work.

PVAs involve the construction of mathematical models that can be used to predict the abundance of populations through time, usually with the aim of measuring the risk of extinction.

As with all models, the models underlying the PVA are only as good as the data used to develop them and the appropriateness of the assumptions made within the model structure.

Since PVA generally requires large amounts of data and these data are often not available, the reliability of viability estimates based on PVA has been questioned in the scientific literature (Beissinger and Westphal 1998, Fieberg and Ellner 2000, Coulson et al. 2001). Therefore, there is inevitably a high degree of uncertainty in using PVA's to estimate extinction risk.

Given this, the Ballina Koala Plan found the following when developing the PVA:

- *Sensitivity analyses are needed for all uncertain parameters*.

  One avenue to deal with the inherent difficulties of determining the parameters for the PVA model is to undertake a large number sensitivity analyses to evaluate the effect of uncertainty in model parameter estimates and to document these effects. A sensitivity analysis was used to determine the variables that will have the most impact on the results and showed which variables were most likely to influence population viability outcomes and if they can be controlled or manipulated by management.

- *The larger the pool of data and expertise contributing to the model, the better the outcome.*

  To ensure that parameters are as robust as possible, input is required from a diverse range of experts in koala ecology, road mitigation, genetics and PVA modelling. Workshopping techniques and arranging for peer comment of critical elements of the PVA was important and underpinned the preparation of the Ballina Koala Plan.

- *Genetic analysis can fill important gaps or strengthen estimates derived by other means.*

  There are a range of parameters within the PVA model where the genetic analysis filled gaps and strengthened estimates derived by other means such as demographic studies based on field survey and literature reviews.

- *PVA results are best used to evaluate different mitigation scenarios.*

  While PVAs are relatively good at predicting the relative impacts of different scenarios, they are less good at predicting absolute levels of time to extinction.

A useful outcome of this PVA analysis is measuring the relative benefits to population viability arising from different mitigation scenarios. The results of the PVA in the Ballina Koala Plan will inform the development of the KMP which sets out how the koalas will be managed as part of the construction of the Upgrade.

2.2 PEER REVIEW

Associate Professor Jonathon Rhodes, the University of Queensland, was engaged by RMS to provide peer review of the Ballina Koala Plan (Appendix 3). Further review and advice on the Plan and its PVA analyses was provided by Professor Kathy Belov and Dr Catherine Grueber, The University of Sydney. The peer review process was conducted in an iterative manner between the reviewers and Dr Kavanagh from Niche Environment and Heritage.

Associate Professor Rhodes found that the report was “scientifically sound” given the limited data and timeframe and is “happy to endorse the contents of that report”. He discusses that “the report details estimates of the potential impact of the road upgrade on the koala population in the region and identifies the extent to which habitat restoration may offset these impacts. It then considers other potential management strategies, which could further compensate for the impact of the road upgrade, by reducing mortality on other roads, or by increasing fecundity through measures such as disease management”.

2.3 MITIGATION MEASURES

The Plan discusses a range of mitigation measures which have been included in the PVA modelling. These include:

- Connectivity structures – RMS in consultations with other agencies and Koala experts, have agreed to build 26 connectivity structures along the 13.5km of Section 10. That will provide about 1.9 connectivity structures per km of new road, or about 1 structure per 520m of the road. (Appendix 3, Figure 5).
- Fencing – the upgrade will be fully fenced with Koala-proof floppy-top fencing. Koala grids will also be placed near intersections. This aims to provide a closed system to prevent road strikes.
- Revegetation – RMS has acquired about 621 ha of forested and cleared land near the upgrade, of which 151 ha is available for revegetation. 130 ha of this has been committed for the planting of koala food trees. According to the Plan this could provide new habitat for about 41 Koalas.

Other potential mitigation and conservation measures are discussed to significantly improve outcomes, include:

- Reducing mortality
    - Fencing of local road hotspots including part of Wardell Road, part of the existing Pacific Highway north of Wardell to Collgardie, Bruxner Highway and Bagotville Road.
    - Reducing predation by domestic dogs
- Increasing fecundity – This could be assisted through reducing the incidence of disease, such as Chlamydia. As part of the survey and capture program undertaken by EcoSure, samples were collected for testing to determine the level of Chlamydial infection in the population. Results from these samples are expected by 4 March 2016 and the results will be considered at that time. Researchers at the University of the Sunshine Coast have developed a vaccine against Chlamydia in koalas. Field trials have shown that it has the potential to protect wild Koalas from infections and improve female reproductive success in females.

Long term monitoring is required to determine whether recovery efforts and mitigation activities have been successful.

2.4 FUTURE VIABILITY OF THE BALLINA KOALA POPULATION

The Plan aims to demonstrate whether the impacts of the road on the koala population are acceptable. No definition of acceptable has been provided. The Plan has interpreted acceptable as meaning no impact.

The Plan demonstrated that the koala population is in decline over the next 50 years with or without the upgrade.

The Plan concludes that if it is assumed no worse than the current status of the population, the highway upgrade, based on a range of scenarios tested, will cause a reduction between 0-9.7% in projected population size over 50 years. However, when mitigation strategies (fencing and revegetation) are included the analysis shows that the upgrade is unlikely to “impact adversely” on the population.

The Plan notes that the “management responsibilities, actions and resources associated with this infrastructure had the potential to arrest the current steep decline in this population”. The Plan shows that intervention is required for this population to ensure its survival due to its current high rates of mortality and low rates of fecundity. This intervention would be required with or without the upgrade.

As discussed above, further mitigation and conservation measures, such as fencing of local road, dog control and Chlamydia vaccination, to improve these rates could further improve the viability of the population.

3 FINDINGS AND NEXT STEPS

3.1 FINDINGS

KEAC has reviewed the Ballina Koala Plan and the large volume of work that has been prepared to inform the Plan, including the PVA.

The findings are as follows:

- *The Ballina Koala Plan is the product of a detailed process*

While there are limitations, including limited data, the Ballina Koala Plan provides a useful framework to understand the drivers of population viability within the Wardell population over time.

- *PVAs provide a range of possible outcomes not a single value*

The models underlying the PVA are only as good as the data used to develop them and the assumptions made in the model structure. Reliable estimates are hard to obtain without many years of data. However while absolute estimates in variability may be unreliable, it has been shown that the relative measures of variability will tend to be more robust. Using PVA to compare the likely relative outcomes of a range of management actions will tend to be a more reliable than using PVA to estimate likely population numbers at a given point in time.

The Plan has looked at the potential outcomes from a range of management scenarios, providing a different result for each scenario. "The impact of the road was estimated to range between no effect and up to a 9.7% decline in the projected population size after 50 years, depending on the uncertainty associated with estimates of the demographic parameters and assumptions about the effectiveness of the connectivity structures that will be provided. In contrast, population projections could be improved substantially through management intervention, including through the provision of supplementary habitat (0.5%) and by a combination of approaches that result in reduced mortality and increased fecundity (potentially up to 496%)."

- *A robust long-term monitoring strategy is required to determine the actual impact of the road*

Associate Professor Rhodes in his review recommended that a robust long-term monitoring strategy be put in place to evaluate the actual impact of the road upgrade and other mitigation approaches and inform future modelling efforts. The studies that were used to inform the PVA could provide a baseline. The outcomes of the monitoring could then be used to inform future decisions related to mitigation and adaptive management of the population.

- *The process of preparing the plan has improved our knowledge of the Wardell koala population*

A significant body of work now exists relating to both the demography and genetic profile of the Wardell koala population. In the case of the genetic analysis, the Wardell population has now been analysed alongside other populations along the NSW coast and the significance the population can now be understood in a larger context. See Appendix 2 and 3 of the Ballina Koala Plan (Appendix 3) for the genetics reports.

- *The future use of PVAs in regulatory decisions warrants further consideration at a policy level in the Commonwealth Department of Environment*

The future use of PVAs in regulatory decisions warrants further consideration at a policy level in the Department of Environment. RMS should offer to share its learnings with the Commonwealth to inform any future application of the PVA technique in regulatory decisions. To this end, it would be useful to further discuss this matter with the Commonwealth at the completion of this process.

KEAC therefore endorses the Ballina Koala Plan and the PVA.

3.2 NEXT STEPS

KEAC will review the Koala Management Plans which will set out the adaptive management and monitoring arrangements for Sections 5, 9 and 10.

A second and final report to the Minister will be prepared following completion of the Koala Management Plans.

KEAC will consider the lessons learnt and priorities for future koala research as part of this report.

REFERENCES

Beissinger, S.R. and Westphal, M.I. (1998) On the use of demographic models of population viability in endangered species management, The Journal of Wildlife Management, 62:821-841

Coulson, T., Mace, G.M., Hudson, E. and Possingham, H. (2001) The use and abuse of population viability analysis, TRENDS in Ecology and Evolution, 16:219-221

Fieberg, J. and Ellner, S.P. (2000) When is it meaningful to estimate and extinction probability? Ecology 26:307-316

APPENDICES

APPENDIX 1 TERMS OF REFERENCE

To provide advice to the Minister for Roads and Freight about:

- The adequacy of the Koala Management Plan prepared for Section 10 in the context of the requirements of the Commonwealth approval conditions.
- Recommendations to improve the Koala Management Plan.
- Any other advice the Committee considers relevant to improve the management of koalas for future road upgrades.

In providing this advice it is expected the Committee will be supported by the RMS and, as necessary, will draw on expertise wider than the RMS for appropriate advice on:

- The appropriate framework to consider the acceptability of impacts on koalas.
- The validity of the scientific methods proposed to undertake population viability modelling for the Ballina koala population.
- Any proposals to manage and mitigate potential impacts on koalas in Ballina as presented in the Koala Management Plans, including the Ballina Koala Plan for the populations as per condition D9 of the NSW Government approval.

Chair and Membership:
The Committee will comprise:

- Professor Mary O'Kane, NSW Chief Scientist and Engineer (Chair)
- Mr Peter Duncan, Chief Executive, Roads and Maritime Services (or delegate)

Two independent experts

- Professor Katherine Belov, the University of Sydney.
- Associate Professor Jonathan Rhodes, University of Queensland.

[The Chair of the committee retains the right to engage additional independent advice to support its considerations.]

Observers
The Committee may invite observers from relevant agencies to attend its meetings.

Quorum
A majority of members of the Committee must participate in Committee meetings.

Meeting frequency and milestones
The Committee is expected to meet to discuss all major milestones in the review process and at other times as decided by the Committee.
Major milestones are the:

- review of the population viability analysis undertaken on behalf of RMS for Section 10 of the Woolgoolga to Ballina Pacific Highway upgrade; and
- review of the Koala Management Plans including the Ballina Koala Plan

Secretariat
Secretariat support will be provided by Ms Julie Ravallion, Senior Environmental Specialist (Biodiversity).

Reporting timeframes
It is expected that the Committee will report to Minister at the completion of each major milestone and provide a final report before 30 December 2015.

APPENDIX 2 CONDITIONS OF APPROVAL

Approval – Pacific Highway Upgrade – Woolgoolga to Ballina (EPBC 2012/6394)

Condition 5 – "In order to ensure the long-term viability of the Ballina Koala population, the approval holder must engage a suitably qualified expert to undertake population viability modeling of the Ballina Koala population over a time period of no less than 50 years, taking into account the impacts resulting from the road upgrade in Section 10. This modelling should consider the current proposed route and any proposed avoidance or mitigation measures as appropriate."

Condition 6 – "The approval holder must have the modelling required by Condition 5 peer reviewed by a second suitably qualified expert."

Condition 7 – "In addition to the Koala Management Plan(s) required by NSW approval conditions D* and D9, to ensure that an unacceptable impact will not occur to the Ballina Koala population, the approval holder must submit for the Minister's approval, a Ballina Koala Plan no less than 3 months prior to commencement of Section 10 of the action, if the impacts to the Ballina Koala population are demonstrated to be acceptable within the Ballina Koala Plan. The Ballina Koala Plan must include:

a. The modelling required by Condition 5 and the results of this modelling, and the peer review required by Condition 6;
b. Discussion of the future viability of the Ballina Koala population;
c. In the context of relevant environmental social and economic considerations, any additional avoidance, mitigation or offsets, beyond those required by the NSW approval conditions, proposed to minimize the impacts to the Ballina Koala population; and
d. Evidence that any additional avoidance and mitigation measures proposed have been considered in the modelling required in Condition 5.

The approval holder must not commence Section unless the Ballina Koala Plan has been approved by the Minister. The approved Plan must be implemented.

NSW State Approval Conditions

D9. As part of the Threatened Species Management Plans required under condition D8, the Applicant shall prepare and implement a **Koala Management Plan** to demonstrate the ongoing survival of the Koala populations at Coolgardie/Bagotville, Broadwater and Woombah/Iluka. The Plan shall be prepared by a suitably qualified and experienced species expert and shall include, but not necessarily be limited to:

a) results of detailed surveys to determine:
(i) the population status of the Coolgardie/Bagotville, Broadwater and Woombah/Iluka Koala populations;
(ii) habitat use and movement patterns of Koala populations within five kilometres of the proposed upgrade, or such area as determined by the independent ecologist; and
(iii) habitat areas likely to be fragmented by the SSI;
including the results of SPOT assessment and radio tracking.
The results and adequacy of surveys shall be verified by an independent suitably qualified and experienced ecologist with appropriate qualifications and experience in Koala and road ecology. Where appropriate, the Applicant may vary the required area of survey specified under condition D9(a)(ii) to the satisfaction of the independent ecologist;

b) (b) a detailed assessment of the impacts to the Koala populations based on the survey results required by condition D9(a), including population impacts and the identification of habitat likely to be fragmented and/or isolated as a result of the SSI;
c) a detailed description, including the location and design, of all proposed avoidance and mitigation measures;
d) justification that the location and design of mitigation measures:
   (i) have been designed with the objective of no Koala road kill from the commencement of construction of the SSI. In the event that a Koala is injured or killed during construction or operation, this shall be reported on the Applicant's website within 24 hours of this occurring, and the record shall remain available for a period of at least five years, unless otherwise agreed by the Secretary;
   (ii) include permanent fencing of the entire SSI for the length of the distribution of the Coolgardie/Bagotville, Broadwater and Woombah/Iluka populations and for two kilometres beyond the distribution of the Coolgardie/Bagotville, Broadwater and Woombah/Iluka population, following the highway or to the nearest natural barrier to Koala movement (e.g. river), after baseline surveys are complete in accordance with condition D9(a) and prior to operation;
   (iii) result in the complete, safe crossing of fauna crossings by the Koala. Fauna crossings shall be provided at a sufficient frequency to ensure that habitat connectivity is maintained or improved from pre-construction conditions, as determined by the independent ecologist and agreed by OEH;
   (iv) provide sufficient opportunities for species dispersal and re-colonisation as determined by the independent ecologist and OEH;
   (v) are in areas that, and are at a sufficient frequency to, achieve (i) - (iv), based on site specific information contained in the survey results required by condition D9(a) and the ecological requirements of the Koala, including but not limited to home range size, local movement patterns and habitat use, in accordance with the advice of the independent ecologist and OEH;
   (vi) all koala underpass structures shall have a minimum height and width of 2.4 metres and a maximum length of 40 metres, or a minimum height and width of 3 metres and a maximum length of 50 metres. The underpass/culvert entrance shall be located at ground level, and no higher in the fill. Structures that provide passage over the road shall have a minimum width of 30 metres and shall be treated with contiguous habitat features;
   (vii) provide passage for Koalas under or over the existing highway (where the existing highway forms part of the SSI) and service roads or local roads (servicing over 100 vehicles per day);
   (viii) effectively minimise the risk of predation from dogs in both dedicated and combined crossings;
   (ix) provide dry passage for dedicated fauna crossings and for combined fauna crossings to the satisfaction of OEH and DoE, at a flood immunity level determined in accordance with condition D2(c)(j);
   (x) provide habitat linkages to crossing structures from adjacent Koala habitat; and
   (xi) ensures that pathways to connectivity structures are not impeded by ancillary facilities, rest areas, service roads or local roads;
e) to be effective to the satisfaction of the Secretary, in consultation with OEH and DoE, provision for the Plan to be revised to include the design and construction of a minimum of one dedicated underpass or land bridge every 500 metres. Underpass structures shall have a minimum height and width of three metres and a maximum length of 50 metres;

f) provision for the installation and vegetation planting of fauna overpasses prior to the commencement of construction;

g) a **revegetation strategy** to be implemented to increase connectivity adjacent to the SSI and leading to crossing locations, and the provision of vegetation planting on land bridges, to ensure the establishment of the vegetation prior to the commencement of construction;

h) details of the proposed monitoring methodology to ensure the effectiveness of the mitigation measures and the ongoing survival of the Coolgardie/Bagotville, Broadwater and Woombah/Iluka Koala populations. Monitoring shall:
(i) include goals that demonstrate the mitigation measures are effective, including clear objectives, milestones, performance measures, corrective actions, and thresholds for corrective actions, and timeframes for completion;
(ii) occur until such time as the mitigation measures are demonstrated to be effective for three consecutive monitoring periods, or as agreed by the Secretary, to the satisfaction of the independent ecologist and OEH; and
(iii) for the purposes of the Coolgardie/Bagotville population, consider the results of the surveys undertaken in the Koala habitat and population assessment: Ballina Shire Council LGA (Biolink Ecological Consultants Pty Ltd, November 2013) in determining the baseline population;

i) where the results of monitoring undertaken in accordance with condition D9(h) suggests that the mitigation measures are ineffective or changes to the population have occurred, the Applicant shall provide the Secretary, within one month of recording the changes, the corrective actions that have been implemented and/or proposed to be implemented, or a procedure for demonstrating that this change is not a result of the SSI. Should the Applicant be unable to demonstrate to the satisfaction of the Secretary that any change to the population is not attributable to the SSI, the SSI shall be deemed as the cause of the impact and the Applicant shall, within one month of these findings, provide, to the satisfaction of the Secretary, in consultation with the OEH and DoE, the proposed corrective actions to address the impacts of the SSI. Any required corrective actions shall include, but not necessarily be limited to:
(i) installation of further crossings or modifications to existing crossings and the provision of evidence of the complete, safe crossing of these fauna crossings by the Koala. Any additional crossings shall be provided at a sufficient frequency to ensure that habitat connectivity is maintained or improved from pre-construction conditions, within two years of their installation; and
(ii) reassessment of all revegetation areas and frequent reporting and maintenance including addressing failures;

j) if the measures in condition D9(i) cannot be demonstrated to be successful within one year of their implementation, procedure for the submission of further offsets in accordance with conditions D5 and D6(j), to be provided within one year of these findings. Further offsets may include:
(i) the legal protection and conservation management of additional areas of existing habitat that actively regenerated and secured into conservation management; and/or
(ii) strategic revegetation of cleared areas to improve connectivity; and/or
(iii) development of a supplementary feeding program and/or breeding program; and/or
(iv) development of a long term predator control program; and

k) evidence of consultation with species experts, OEH and DoE in addressing the requirements of this condition, and demonstration of how comments provided by the species experts, OEH and DoE, as a result of this consultation, have been addressed.

The Koala Management Plan shall be submitted and approved by the Secretary prior to the commencement of construction of the relevant stages of the SSI. The approved Koala Management Plan shall be implemented prior to the commencement of construction of the relevant stages.

APPENDIX 3 BALLINA KOALA PLAN

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Environment and Heritage


Ballina Koala Plan

Koala Population Viability Analysis of the proposed Pacific Highway Upgrade near Wardell, NSW

Prepared for NSW Roads and Maritime Services

January 2016

Document control

Project no.: 2266

Project client: NSW Roads and Maritime Services (RMS)

Project office: Sydney

Document description: Population Viability Analysis for the Ballina Koala population, considering the proposed section 10 W2B upgrade.

Project Director: Dr Rhidian Harrington

Project Manager: Dr Rod Kavanagh

Author: Dr Rod Kavanagh

Internal review:

Document status:

Document address:

Local Government Area: Ballina

Document revision status

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Cover photograph: Koala living in Broad-leafed Paperbark and Swamp Mahogany forest; and, the study area, showing the route of the proposed Pacific Highway Upgrade in Section 10, west of Wardell.

Executive summary

Context

The Australian Government Conditions of Approval for Section 10 of the Pacific Highway Upgrade near Wardell in northern NSW require the approval holder to demonstrate that the impacts to the Ballina Koala population as a result of the proposed highway upgrade are acceptable over a 50 year period. A population simulation and threat modelling process (Population Viability Analysis) was the method specified to undertake this assessment. A local Koala ecological and population demographic study, and two genetics studies, were commissioned by the Roads and Maritime Services to provide the parameter estimates needed to run the PVA. A range of mitigation options was also available for assessment.

Aims

To estimate the likely impact of the proposed highway upgrade in Section 10 on Koala population viability over a 50 year period, and over a range of plausible management scenarios.

Methods

The VORTEX (version 10) PVA software program was used to conduct the analyses. All population size, distribution, demographic and stochastic inputs to the model, as well as the frequency of likely catastrophic events, were provided by the authors of the local Koala field study, which included details for 50 captured animals. The genetics studies were used to estimate the minimum numbers of Koalas immigrating into and emigrating from the study area because the distribution of habitat in the region showed that the population was not "closed". These studies were also used to estimate the minimum numbers of animals dispersing, and the extent of inbreeding, within the study area. The impact of the proposed road was assessed by comparing population projections based on differences in the rate of dispersal between two sub-populations as influenced by the proposed connectivity structures. The provision of supplementary habitat for Koalas in the study area was modelled through an increase in the projected carrying capacity of the habitat. Management options were investigated by varying the levels of key population parameters.

Key results

Population projections showed a gradual decline over 50 years, with or without the proposed highway upgrade. The impact of the road was estimated to range between no effect and up to a 9.7% decline in the projected population size after 50 years, depending on the uncertainty associated with estimates of the demographic parameters and assumptions about the effectiveness of the connectivity structures that will be provided. In contrast, population projections could be improved substantially through management intervention, including through the provision of supplementary habitat (0.5%) and by a combination of approaches that result in reduced mortality and increased fecundity (potentially up to 496%).

Conclusions and Management implications

The projected population decline was due to births not being adequate to offset deaths, regardless of the presence of the highway upgrade. Any efforts to increase fecundity and/or reduce mortality in the region will improve population viability.

Significant opportunities exist to reduce Koala mortality by the provision of a range of mitigation structures, including Koala-proof fencing along the proposed highway upgrade and other roads in the area, and other management interventions. Community involvement is needed to control dog predation and to support trials of a new *Chlamydia* vaccine to increase fecundity.

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1. Introduction

1.1 Context

The NSW and Australian Governments are upgrading the Pacific Highway between Woolgoolga-Ballina (155 km) as part of State Significant Infrastructure. This Project is divided into 11 Sections, each of which has been subject to ecological surveys to determine the likely effects of the proposed upgrade on flora and fauna. The Koala in NSW is listed as ‘vulnerable’ under the Threatened Species Conservation Act 1995 (TSC Act), and also as ‘vulnerable’ under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act). The present study incorporates the results of surveys and other assessments for the Koala in Section 10.

The Koala population located in Section 10, between Bagotville and Coolgardie west of Wardell, has been proposed by Phillips and Chang (2013) as suitable for listing as an “important population” under the EPBC Act. This population is referred to specifically in the draft Comprehensive Koala Plan of Management (2015) for the Ballina Shire Local Government Area and in the Conditions of Approval for the Project.

1.2 Conditions of Approval

On 14 August 2014, the Australian Minister for the Environment approved the Pacific Highway Upgrade from Woolgoolga to Ballina, NSW, subject to conditions, including Conditions 5, 6, 7, 8, 9 and 10 that refer specifically to the Koala.

Condition 5 states: “In order to ensure the long-term viability of the Ballina Koala population, the approval holder must engage a suitably qualified expert to undertake **population viability modelling** of the Ballina Koala population over a time period of no less than 50 years, taking into account the impacts resulting from the road upgrade in Section 10. This modelling should consider the current proposed route and any proposed avoidance or mitigation measures as appropriate”.

Condition 7 states: “In addition to the Koala Management Plans(s) required by NSW approval conditions D8 and D9 (approved by the NSW Minister for Planning on 24 June 2014), to ensure that an unacceptable impact will not occur to the Ballina Koala population, the approval holder must submit for the Minister’s approval a **Ballina Koala Plan** no less than 3 months prior to the commencement of Section 10. The Minister will only approve the plan and the commencement of Section 10 of the action if the impacts to the Ballina Koala population are demonstrated to be acceptable within the Ballina Koala Plan. The Ballina Koala Plan (this document) must include:

a. The modelling required by Condition 5 and the results of this modelling, and the peer review required by Condition 6;
b. Discussion of the future viability of the Ballina Koala population;
c. In the context of relevant environmental and economic considerations, any additional avoidance, mitigation or offsets, beyond those required by the NSW approval conditions, proposed to minimise the impacts to the Ballina Koala population; and
d. Evidence that any additional avoidance and mitigation measures proposed have been considered in the modelling required in Condition 5.

The approval holder must not commence Section 10 unless the Ballina Koala Plan has been approved by the Minister. The approved Plan must be implemented”.

This Ballina Koala Plan is an essential component of the overall strategy to minimise and mitigate the impacts to the Ballina Koala population within Section 10 of the proposed Pacific Highway Upgrade.

1.3 Project objectives

The objectives of this project and report are to satisfy the Australian Government's Conditions of Approval in relation to preparation of the Ballina Koala Plan. This includes the requirement to:

- estimate the likely impact of the proposed highway upgrade in Section 10 on Koala population viability over a 50 year period; and to
- investigate the relative benefits of a range of plausible management scenarios that could be implemented by RMS to minimise any potential impacts of the proposed highway upgrade.

2. The Study Area

2.1 Study area

The Koala population located in Section 10, which extends 13.5 km north of the Richmond River and includes the localities of Bagotville and Coolgardie west of Wardell, is the subject of this study. This population has been proposed by Phillips and Chang (2013) as suitable for listing as an "important population" under the EPBC Act. The area nominated as enclosing this population is approximately 8,250 ha, the boundaries of which are displayed in Figure 1. This population is not considered as "closed" for the purposes of modelling because of the degree of habitat connectivity with surrounding areas (Figure 1).

The general landscape context within the study area is a predominantly-cleared, sometimes waterlogged, fertile valley surrounded to the west by mostly tall forested lands on slopes and ridges along the Blackwall Range, and to the east by low slopes covered mostly by drier forests and woodlands with large areas of tall heathland growing on less fertile soils (Figure 1). The valley and lower slopes have been used extensively for grazing and sugar cane production, although significant areas of remnant or regrowth native vegetation still remain, particularly along watercourses. The proposed route of the highway upgrade in Section 10 traverses through the eastern side of the valley and will result in the loss of a further 34 ha of native vegetation, half of which (17 ha) is recognised as good habitat for Koalas (Table 1).

2.2 Geology

Five main geological types are present in the study area (Figure 2). These geological types are ranked in approximate order of the fertility of the soils derived from them:

- Basalt (Tllb)
- Meta-basalt (Cnx)
- Undifferentiated alluvial deposits/floodplain and swamp deposits (Qa)
- Coarse-grained conglomerates (Rjbwx)
- Dune sand and sand sheets (Qb)

2.3 Vegetation types

A range of remnant or regrowth native vegetation types is present in the study area (Figure 3). The vegetation types likely to be of most importance to Koalas, due to the expected presence of Koala food tree species within them, include:

- Paperbark (depending on the proportion of Swamp Mahogany *Eucalyptus robusta*);
- Lowland Red Gum;
- Wet Flooded Gum – Tallowwood;
- Foothill Grey Gum – Ironbark – Spotted Gum;
- Northern Open Grassy Blackbutt

Other vegetation types present in the study area are less likely to make a significant contribution to Koala habitat but may contribute to Koala dispersal and habitat connectivity in the region.

Study Area

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FIGURE 1

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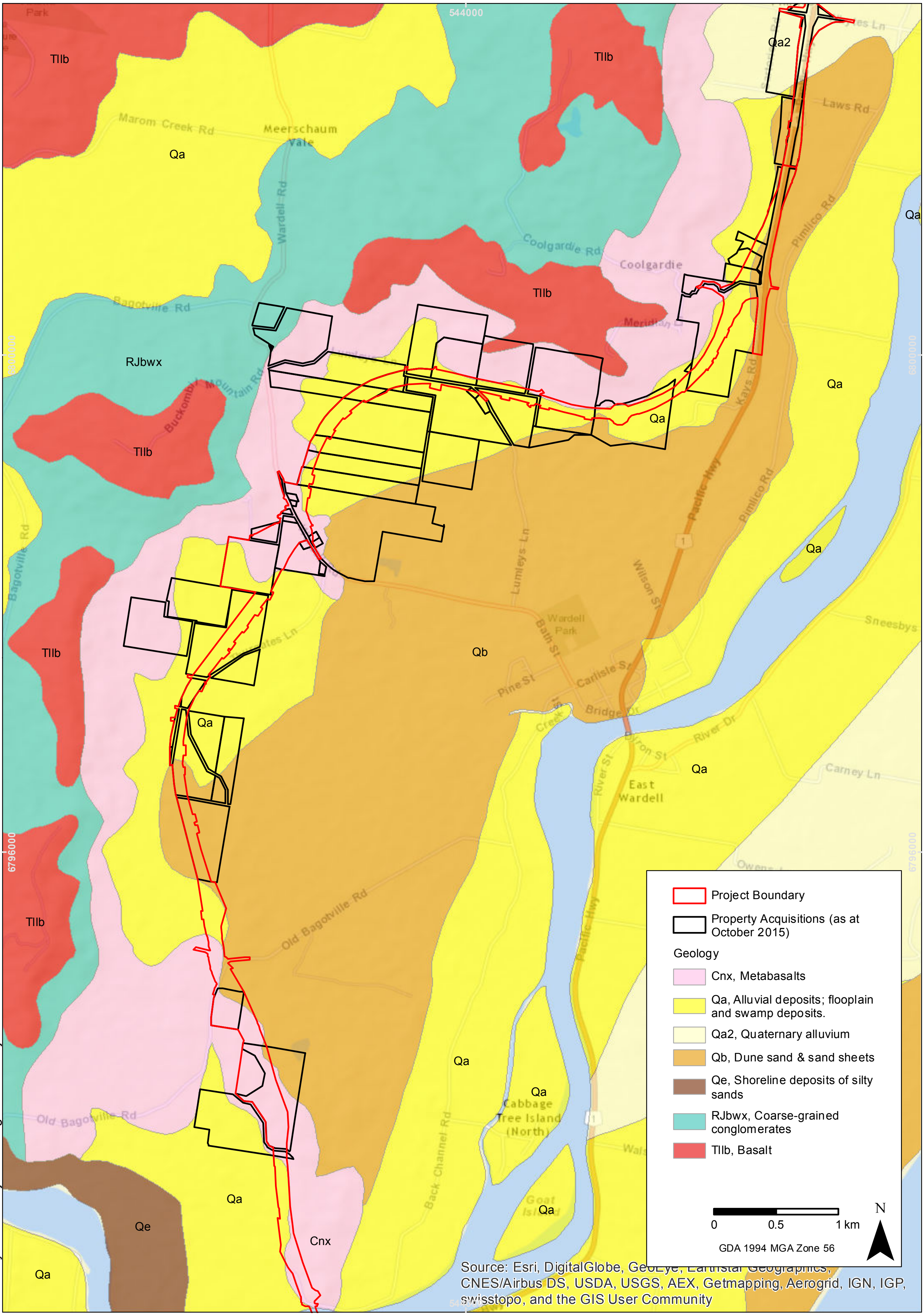

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Section 10 - Geology

FIGURE 2

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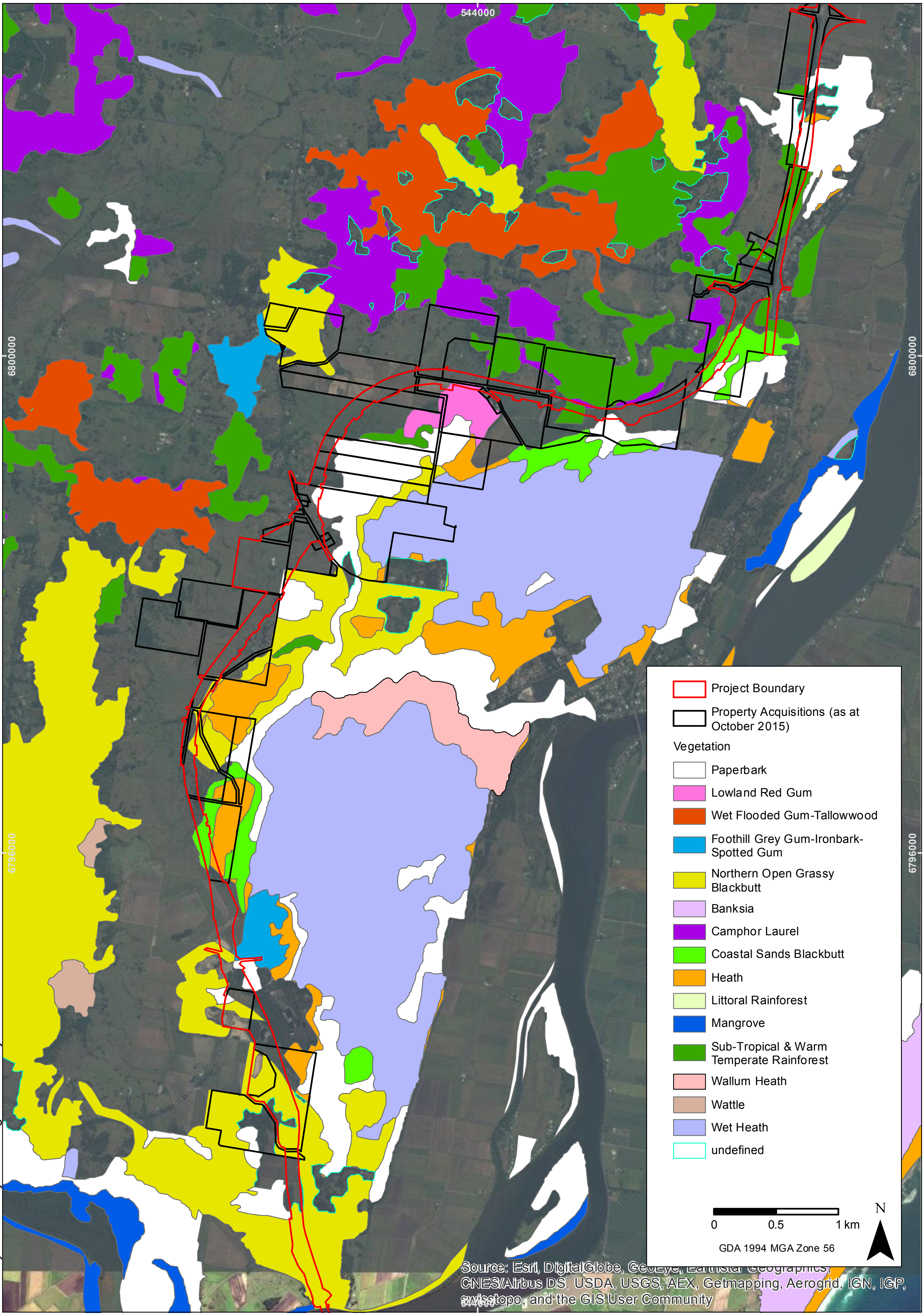

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Section 10 - Vegetation type

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FIGURE 3

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Table 1: Koala habitat quality scores (EPBC Act 1999 Environmental Offsets Policy Calculator) and the areas (ha) of each Biometric Vegetation Type occurring within the clearing "footprint" of Section 10 of the proposed Pacific Highway Upgrade between Bagotville and Coolgardie near Ballina. Source: Pellow and Semeniuk (2015).

Koala Habitat Quality Score Vegetation Types in Section 10 clearing "footprint"	0	1	2	3	4	5	6	7	8	9	10	Total
Blackbutt- Pink Bloodwood Shrubby Open Forest					0.05	2.21	0.14	2.97				5.38
Blackbutt Grassy Open For.							0.23	0.93	0.91		1.53	3.60
Cinnamomum camphora				1.44								1.44
Cleared	115.02				0.14			0.93				116.09
Mangrove-Grey Mangrove Low Closed Forest	0.17											0.17
Narrow-leaved Red Gum Woodlands						1.06	2.01	0.70		1.26	3.92	8.94
Paperbark Swamp Forest	0.00			0.41	0.88	1.61	2.17			0.25	0.64	5.96
Scribbly Gum-Needlebark Heathy Open Forest						1.98	1.47					3.45
Swamp Mahogany Swamp Forest						0.63	0.27		1.23			2.12
Tuckeroo-Riberry-Yellow Tulipwood Low Rainforest	0.01				0.19	0.89						1.09
White Booyong-Fig Subtropical Rainforest				0.39	0.52	0.47		0.53				1.92

3. Population Viability Analysis

3.1 Definition and purpose

Conservation problems are almost always multi-faceted, involving not only complex dynamics of biological populations, but also interactions with human populations. Many people need to contribute knowledge, expertise, and ideas in order to achieve the recovery of threatened species. Population viability analysis (PVA) can provide a framework for incorporating the many needed kinds of knowledge into species conservation efforts because PVAs provide a means for assessing the relative contributions of factors that can threaten the persistence of populations (Lacy 1993, Lindenmayer *et al.* 1993). Compared to other alternatives for making conservation decisions, PVA provides a rigorous methodology that can use different types of data, a way to incorporate uncertainties and natural variabilities, and products or predictions that are relevant to conservation goals (Akçakaya and Sjögren-Gulve 2000).

Shaffer (1981) first defined a minimum viable population (MVP) as the size at which a population has a 99% probability of persistence for 1000 years, but it has become more meaningful biologically to consider it to be the size below which a population's fate becomes determined largely by the range of stochastic factors that threaten its existence (Soulé 1987). In simple terms, small populations are more vulnerable to extinction than large populations because unexpected catastrophic events can lead to the death of all individuals, however, even large populations are threatened over time if birth rates are insufficient to offset death rates (Caughley 1994). There is no true consensus on a definition of the term PVA, with previously used definitions ranging from qualitative, verbal processes without models, through to mathematically-sophisticated, spatially-explicit, stochastic simulation models (Reed *et al*. 2002), while numerous practitioners have suggested that the latter definition be used (Ralls *et al.* 2002) and, with the availability of population-modelling software, this has become the most common approach.

The general concept of 'island biogeography' (see Diamond and May 1976) applies to the management of wildlife populations. As wildlife populations become smaller, additional threats to stability and persistence arise, which from a certain point forward, may be difficult to reverse (Lacy 2000b). These problems of small populations usually arise from stochastic processes (Lacy 2000b). Many aspects of population biology are 'sampling processes', such as breeding success, transmission of genetic alleles, survival and dispersal (Lacy 2000b). Uncertainty arises from their outcomes, leading to instability in population dynamics (Lacy 2000b). Demographic stochasticity is the random variation in deterministic factors, such as the numbers of births, deaths and sex ratio in a population, that result from the fates of individuals being outcomes of probability-based events (i.e. reproduction, mortality and sex determination; Shaffer 1981). The function of a PVA model is to assess, over a defined time period, the relative contributions of all deterministic factors and variations in demographic stochasticity towards long-term population persistence. This process can identify those variables which are likely to have the greatest influence on population outcomes, and accordingly which variables should be targeted for management attention should the projected population increase, or decrease, beyond acceptable limits.

3.2 Brief review of PVAs

Simulation-based PVAs (compared to a qualitative PVA) use computer software to model (predict) population trends for a single species, over time. The PVA process relies upon the availability of accurate demographic data for the population being modelled. Common software used for simulation-based PVA includes ALEX (Analysis of the Likelihood of Extinction; Possingham and Davies 1995), RAMAS (Applied Mathematics, NY, USA) and VORTEX (Lacy 1993). These programs have been reviewed and found to be appropriate for a wide range of applications (Lindenmayer *et al*. 1995).

In recent times a number of code-based models have been developed, such as ‘PVAClone’ in the statistical package R, while others have completed PVA analyses using transition matrix models (Edwards *et al*. 2015) and simulation modelling software such as E-Surge (Choquet *et al.* 2009, Hernández-Matías *et al*. 2015). In general PVAs may consider the following types of scenarios (as examples):

- Assess the effectiveness of different control measures for pest animals (Rømer *et al*. 2015).
- Management and recovery of threatened species (Bode and Brennan 2011, Lindenmayer and Possingham 1995, Taylor and Goldingay 2013).
- Competition with introduced species (Glen and Dickman 2013).
- Assessment of the potential success of re-introductions (King *et al*. 2014).
- Future trends in populations, particularly in relation to disturbance regimes (Lunney *et al*. 2007, Lindenmayer and Possingham 1995).

3.3 Strengths and weaknesses of PVAs

Modern applications of PVA are not intended to provide a definitive statement about population size after a specified time period. Instead, the value of this technique is to provide an opportunity for interested parties to assemble relevant information about the status of a population and likely threats to its persistence. The technique is used primarily to evaluate the relative impacts of alternative management scenarios that can be used to inform management actions (Beissinger and Westphal 1998, Coulson *et al*. 2001).

There have been few empirical studies that have attempted to verify the accuracy of PVAs. Brook *et al*. (2000) retrospectively examined the long-term population trends for a range of species based on 21 long term studies in which the actual population sizes were known. They found that the PVA predictions were relatively accurate, with population size estimates not differing substantially between the predicted and observed numbers (Brook *et al*. 2000). However, this level of optimism was criticised by Coulson *et al*. (2001) who cautioned that, while PVAs could be useful for comparing the consequences of different management or conservation strategies, the lack of long-term demographic data and confidence in the future (e.g. impact of catastrophes, habitat availability) would preclude accurate projections of the status of most wild populations. Lindenmayer and McCarthy (2006) used data collected over a seven year period to examine changes in populations of arboreal marsupials in timber production forests. They found that, while many variables were difficult to estimate and contributed to some variation, the overall PVA model was generally consistent with field observations.

One of the biggest influences on the outcome of a PVA is the size of the study area in which populations are being modelled. This is because of the strong relationship between population size and its resilience to the factors which may lead to its extinction; it is well known that smaller populations are much more likely to become extinct than larger populations, all else being equal (Caughley 1994). Thus, when assessing the impact of a development, it is important to ensure that the study area is not so large that most individuals in the population will have no interaction with the proposed development, but not so small that the population is likely to become extinct anyway due to the “small population paradigm” (Caughley 1994).

The main weakness of the PVA process is determining the overall accuracy of the input data (Beissinger and Westphal 1998, Coulson *et al*. 2001). If the input data over or underestimates particular parameters, then the final output will report erroneous results. Any model is only as good as the data which are used, and it is important to be cautious in the interpretation of results (Lindenmayer *et al*. 1995, Beissinger and

Westphal 1998). Often it is recommended to obtain long-term data over many years, in an attempt to calibrate the PVA model (see Lindenmayer and McCarthy 2006).

The strength of the PVA process is it can be used to construct and inform management decisions, to determine if different actions are likely to result in a positive or negative effect on a particular population. The PVA process includes a prediction of population size, thus well designed monitoring programs can be used to test the original predictions from the PVA.

3.4 Examples of the use of PVA

Mammals in Australia

Population viability analysis has been completed for a range of mammal species in Australia, investigating a range of research and management questions. These include the minimum viable area for the Yellow-bellied Glider (*Petaurus australis*; Goldingay and Possingham 1995), the effect of urban fragmentation and the use of connectivity structures for the Squirrel Glider (*Petaurus norfolcensis*; Taylor and Goldingay 2013), and competition between Foxes (*Vulpes vulpes*) and the Spotted-tailed Quoll (*Dasyurus maculatus*; Glen and Dickman 2013). In the Central Highlands of Victoria, several PVAs have been completed for the Leadbeater's Possum (*Gymnobelideus leadbeateri*) in relation to different disturbance regimes (i.e. logging and burning; Lindenmayer *et al.* 1993, Lindenmayer and Possingham 1995, Lindenmayer and Possingham 1996).

The Koala

Three publications, covering PVAs for four Koala populations have been completed (Table 2). These studies have considered both large and small population sizes, ranging from 20 to 800 individuals. Populations considered include the Iluka population, located to the southeast of this study area (Lunney *et al.* 2002), the Port Stephens population (Lunney *et al*. 2007), one population in southeast Queensland and another in Central Queensland (Penn 2000). All of these studies used Vortex to model changes in the Koala populations of those areas.

Three of the four Koala populations examined showed evidence of a significant decline, due primarily to high mortality and sometimes low fecundity. In all modelled scenarios, attempts to reduce mortality had more influence on population viability than any other factor. None of these studies explicitly examined the role of vehicle collisions, although this factor was recognised as one of several that may be contributing to high rates of population mortality. In south-east Queensland, Rhodes *et al*. (2011) reported on the separate effects of an array of threatening processes leading to the decline of a large population of Koalas, and road mortality was a significant factor.

Table 2: Input values for key variables used in previously published PVAs for the Koala.

Variable	Iluka (Lunney *et al.* 2002)	Oakey (SE Queensland; Penn 2000)	Springsure (Central Queensland; Penn 2000)	Port Stephens (Lunney *et al.* 2007)
Initial population size	20	46	20	800
Stable age structure at start	Yes, calculated by Vortex			Yes
Initial population gender structure	13F, 7M			
Maximum age	12	12	12	12
Minimum female breeding age	2	2	2	2
Minimum male breeding age	3	3	3	3
Sex ratio (% male)	55%	57%	53%	53%
% litter size 1	20% (±10%)	57% (±17.85%)	31% (±15.61%)	77% (7%)
% litter size 0	80%	43% (±17.85%)	69% (±15.61%)	33%
% males in breeding pool	50%	50%	50%	100%
Female mortality age 0	32.5% (±3.25%)	32.5% (3.25%)	30% (3%)	40% (4%)
Female mortality age 1	17.3% (±1.73%)	17.27% (1.727%)	15.94% (1.594%)	40 (4%)
Female mortality adult	9.2% (±0.92%)	9.17% (0.917%)	8.47% (0.847%)	23 (2.3%)
Male mortality age 0	20% (±2%)	20% (2%)	20% (2%)	40% (4%)
Male mortality age 1	23% (±2.3%)	22.96% (2.296%)	22.96% (2.296%)	40% (4%)
Male mortality age 2	23% (±2.3%)	22.96% (2.296%)	22.96% (2.296%)	40% (4%)
Male mortality adult	26.4% (±2.64%)	26.36% (2.636%)	26.36% (2.636%)	39% (3.9%)
Density dependence	Nil	Nil	Nil	Nil
Probability of catastrophe	3%	5%	5%	10%
Severity on reproduction	50%	55%	55%	5%
Severity on survival	50%	63%	63%	5%
Inbreeding depression	Nil	Nil	Nil	Nil
Environmental variation, survival and reproduction	Concordant	Concordant	Concordant	Concordant
Carrying capacity (K)	50	70 (7)	60 (6)	2500
Harvest	Nil			Nil
Supplementation	1 male, age 2 per annum	Nil	Nil	Nil

4. Vortex software

4.1 Vortex

VORTEX is an individual-based simulation program that models the effects of mean demographic rates, demographic stochasticity, environmental variation in demographic rates, catastrophes, inbreeding depression, harvest and supplementation, and metapopulation structure on the viability of wildlife populations (Lacy 2000a).

Vortex has been reviewed on many occasions in relation to other computer software packages, but it is important to remember that any computer program necessarily contains a large number of assumptions and simplistically models the behaviour of animals. Thus, the results of viability analyses can only be estimations of the actual dynamics of wild populations. As a result, caution should be used when interpreting and applying the results of any such analyses (Lindenmayer *et al*. 1995).

4.2 Assumptions and limitations

In their simplest form, population viability analyses assume that the population being modelled is a "closed" population (e.g. Penn 2000, Lunney *et al*. 2007), with no immigration in, and no emigration out, however this is rarely the case and Vortex contains the flexibility to take this into account if the information is available. The landscape context in the study area, with its fragmented forests and woodlands set in, or surrounded by an agricultural matrix, is clearly "porous" to Koala movements (Neaves *et al*. 2015, Norman *et al*. 2015) and, therefore, it is important to account for immigration and emigration (this was done in the present study using the "supplementation" and "harvest" features, respectively, of the program).

Vortex requires an assessment of whether inbreeding depression (i.e. the reduction in fitness of offspring produced by inbred mating) is present in the population being modelled and, while this can have a significant effect on results, the information is rarely available for wild populations. Some authors caution not to disregard the influence of inbreeding depression on extinction risk (O'Grady *et al*. 2006) as it may lead to serious overestimates of the survival prospects of threatened taxa, so the presence of inbreeding depression has been assumed for this population.

However, inbreeding may or may not lead to inbreeding depression, as the latter depends on the numbers and types of lethal alleles that are present in the population and whether matings are random in populations of at least moderate size. The Ballina Koala population was reported to have high levels of genetic diversity with very low levels of inbreeding (Neaves *et al*. 2015, Norman *et al*. 2015). Unfortunately, nothing is known about the number of lethal alleles that are present in this population, or the extent to which free-ranging wild populations are likely to be impacted. Again, based on the recommendations of O'Grady *et al*. (2006), we assumed the likely presence of lethal alleles in the population and have accepted the Vortex default value of 6.29 lethal alleles, although a range of values was also considered in this study. Accordingly, in the current study, all scenarios were modelled with inbreeding depression and the presence of lethal alleles. This had the effect of reducing population size projections by approximately 20-40% of those estimated when inbreeding depression and the presence of lethal alleles was switched "off" in the analyses.

Population viability analyses require accurate demographic data and accurate measures of the variability in these data over months and years. Most studies, including this one, take snapshot samples of population demography within usually one year or season, and hope that this information is representative of the population of interest. Of course, the incidence of drought, disease, predation and many other factors can combine to ensure that once-only samples can be unrepresentative, leading to misleading results.

One of the most difficult aspects of PVA using Vortex is to estimate the year-to-year variability associated with the mean values that are calculated for most variables (Beissinger and Westphal 1998). Without large sample sizes and extended periods of data collection, variability is difficult to calculate yet inaccurate measures can have a large effect on results.

PVAs also require an accurate understanding of population size and its distribution. If population size is under or overestimated, an inaccurate model will be produced.

Population dispersal rates and the success of these movements (i.e. percent survival) between sub-populations are crucially important in modelling the effects of a major highway bisecting this population yet, while Koalas have been observed using them, the effectiveness of the proposed connectivity structures is not well known.

4.3 Structure and inputs

Vortex requires data inputs for numerous variables in multiple categories. These inputs are usually means, as well as the variation around these means that is caused by environmental and annual fluctuations. However, environmental and annual variation cannot usually be estimated from short term studies (see above). Instead, most studies, including this one, incorrectly substitute the standard deviation around the mean for one particular year as the best available input for the Environmental Variation (EV) that is associated with each parameter estimate, given that we have no idea of the inter-year variability of these estimates. The principal categories of information requiring inputs in Vortex are:

- Scenario settings
- Inbreeding depression and number of lethal alleles
- Dispersal
- Reproductive system
- Reproductive rates
- Mortality rates
- Catastrophes
- Mate monopolisation
- Initial population size
- Carrying capacity
- Harvest rates
- Supplementation rates
- Genetics

A summary of the key inputs required for Vortex are presented in Table 2 (based on other studies) and Table 3 (this study).

5. Sources of Information for Modelling

5.1 Ecosure/Biolink study

An area of approximately 8250 hectares has been nominated as one encompassing an “important population” of Koalas in the Ballina Local Government Area (Phillips and Chang 2013, Phillips *et al*. 2015), and this was selected as the focal area for this study. The study area is located in the Ballina Local Government Area and is known locally as the Blackwall Range to the west and north of the proposed alignment and as Wardell heath to the east. The eastern and southern boundary of the study area is the Richmond River, with Sugar Cane plantations predominating in the east (Figure 1). Some forest connectivity occurs to the west and north, with rainforest vegetation occurring to the north (Figure 3). The landscape context is porous to Koala movements, with Koalas living adjacent to the study area and elsewhere in the LGA.

Population distribution and habitat occupancy

Within the 8247 ha study area, 2152 hectares was estimated to contain Preferred Koala Habitat (PKH), including 96 hectares of Primary Koala Habitat (Phillips *et al.* 2015; Appendix 1). Mean Koala population densities typical of the vegetation types present in the study area were estimated from existing data collected in the region by Phillips *et al*. (2015). Koala population density was estimated from observations of live animals encountered on 1 ha sampling plots distributed throughout the study area.

Sampling procedure for demographic determinations

A 2.5 km x 2.5 km grid square was applied across the study area, where up to seven Koalas were sampled from any one grid square. When Koalas were encountered, they were captured using either flagging or the fence-trap method. Once on the ground, Koalas were anesthetised, with the animal’s gender, weight and body condition score recorded. The reproductive status of captured females was assessed using a four tier system consisting of 1/ no pouch young present, nor evidence of recent lactation; 2/ pouch young present; 3/ back young present; 4/ neither pouch young or back young present, but evidence of recent lactation. Tooth wear classes (after Gordon 1991) were determined, ranging from TWC 2-6.

A total of 40 Koalas was captured using the flagging or fence-trap method, while a further two were captured by hand when they were observed in the open. Another nine Koalas were found deceased in the study area, with three being from dog/fox attack and six as a result of vehicle strike. Koalas sampled were broadly distributed throughout the study area, however, some aggregations did occur in the southern part of the study area. Ocular and urogenital swabs, fur samples and ear tissue were also taken from each captured animal, in order to undergo various pathological and genetic analyses. The results of these data were unavailable at the time of the present study.

Of the 30 female Koalas sampled, 13 showed evidence of reproduction, distributed between Tooth Wear Classes 3-5, resulting in a reproductive rate of 43.33% (SD= 9.2%) which was later updated to 44.83% (SD=9.27) (Phillips *et al.* 2015; Appendix 1). Overall annual mortality was estimated at 9.94% (SD= 8.91%), however this average estimate varied greatly among age classes (Table 3), due to an unusual age-class distribution in the population.

Catastrophes

The likely distribution, frequency and severity of drought and fire on Koala survival and breeding success were reported in Phillips *et al*. (2015).

Table 3: Input values for key variables used in this study (primary source: Phillips *et al.* 2015).

Variable	Values used in this study
Initial population size	Total 236 individuals (includes estimated number of 0-1 year old males and females, based on fecundity of breeding females – otherwise 196 individuals, 125 females and 71 males) Individuals distributed as 180 west and 56 east of the proposed road upgrade.
Stable age structure at start	No. Local baseline "snapshot" field data used.
Initial population gender structure	0.64F: 0.36M, but 50:50 at birth
Maximum age	10
Minimum female breeding age	2
Maximum female breeding age	8
Minimum male breeding age	4
Sex ratio (% male)	36%
% litter size 1	44.83%
% litter size 0	56.66%
% males in breeding pool	76.47%
Female mortality age 0	19.7% (±11.63%)
Female mortality age 1	19.7% (±11.63%)
Female mortality adult	7% (±4.421%)
Male mortality age 0	19.45% (±11.49%)
Male mortality age 1	19.45% (±11.49%)
Male mortality age 2	30.56% (±18.05%)
Male mortality age 3	4.3% (±2.54%)
Male mortality adult	4 (±2.525%)
Density dependence	Nil (unknown)
Probability of catastrophe over 50 years	Drought 21% Fire 3%
Severity on reproduction	Drought effects restricted to upper slopes (32% of Koala habitat) in the study area. Severity: reduction of reproductive output by 15% in drought years. Fire effects restricted to 10% of the study area. Severity: reduction of reproductive output by 15%.
Severity on survival	Drought causes no additional mortalities. Fire results in 40% mortality of all individuals living within the fire boundary (i.e. 10% of the population).
Inbreeding depression	Modelled with inbreeding depression. Number of lethal alleles set to default 6.29.
Environmental variation, survival and reproduction	Concordant; i.e. good years for reproduction also typically good for adult survival.
Dispersal	Modelled for two sub-populations. Levels set to 3.95 individuals (1.98 each way) per year (see section 5.4), as well as other plausible values (0.792, 4, 8, 10, and 20 individuals each way per year).
Carrying capacity (K)	Population carrying capacity set at 291 (±15) individuals (approximately half of the available habitat remains unutilised). For sub-populations, 222 (±15) individuals distributed in the west and 69 (± 15) in the east. Replanting of 130 ha of new habitat for Koalas was modelled by gradually raising the carrying capacity by three animals per year beginning at year 7 (i.e. to a maximum of 25 and 16 animals for each sub-population) over a 15 year period, after accounting for the potential loss of 5 animals due to habitat clearing during road construction.
Harvest	The effects of mortality due to vehicle strike and dog attack in the study areas are already included in the mortality estimates. However, because of the porous boundaries of the study area, 2.85 individuals (60%M:40%F) (see section 5.5) were permitted to emigrate from the study area each year
Supplementation	Similar to above, 2.85 (60%M:40%F) (see section 5.5) individuals were permitted to immigrate into the study area each year.
Genetic inputs	Initial allele frequencies were entered using a spreadsheet provided by Dr C. Grueber (Sydney University) based on the results of the two genetics studies. 30 neutral loci modelled. Additional loci only included in summary statistics.

5.2 Interpretation and use of parameter estimates

All population size, distribution, demographic and stochastic inputs to the PVA model, as well as the frequency of likely catastrophic events (i.e. the “baseline” model), were provided by the authors of the local Koala field study, which included details for 50 captured animals (Phillips *et al*. 2015; Appendix 1). It is unknown whether the “baseline” demographic parameters, collected from this once-only snapshot sample, are truly representative of the population.

Population demography

The age-class and gender distribution of the Koala population sampled by Phillips *et al.* (2015) contained a number of unexpected results. Firstly, the sex ratio of the sample was highly biased towards females (64%). Secondly, the proportional representation within the population of young females and males in the 2-3 year old age-classes was unusually small, suggesting very high mortality of young Koalas. Thirdly, breeding success per year among adult females was relatively low (44.83%), and was restricted to females occurring within age-classes 3-7 years. For a species with an expected lifespan of approximately 10 years, the observed low breeding success suggests one of the following: that environmental conditions were not favourable to the population during the years prior to sampling; that habitat quality was not as good as expected; that disease may be a factor limiting reproductive output; and, that high mortality could be accounting for the relatively few older-aged, potentially-breeding, animals in the population. The causes of the observed demographic “imbalance” are unknown. However, mortality due to vehicle-strike in the study area appeared to be relatively high and clinical signs of disease appeared to be relatively low (Phillips *et al*. 2015).

Population size

Population estimates provided by Phillips *et al*. (2015) were based on an (unmapped) assessment of the distribution and amount of habitat (i.e. 2152 ha) considered as “preferred” by Koalas in the study area. The results of two surveys, conducted 2 years apart, were pooled to estimate Koala population size occurring within the area of preferred habitat. In the first survey, one Koala was observed in diurnal searches of 42 x 0.2 ha plots (8.4 ha sampled), providing a population density estimate of 0.12 ± 0.05 (SD) Koalas per ha or 259 ± 107 Koalas in the study area. In the second survey, three Koalas were observed in diurnal searches of 46 x 1 ha plots (45.34 ha sampled), providing a population density estimate of 0.066 ± 0.037 (SD) or 142 ± 80 Koalas in the study area. When the results of these two surveys were pooled, Phillips *et al*. (2015) estimated 196 ± 65 (SD) Koalas in the study area which, based on the skewed sex ratios observed in the demographic study (above), translated to a population comprised of 125 females and 71 males.

In the PVA model, we used an initial population size of 236 which included an additional 40 animals that we calculated to form the un-sampled 0-1 year age-class (TWC 1), based on the estimated numbers and breeding success of adult females aged 3-7 years.

Carrying capacity

As indicated above, the amount and distribution of habitat for Koalas in the study area was unmapped, but estimates from previous studies in the region, and elsewhere, were used by Phillips *et al*. (2015) to classify 2,152 ha into four Koala habitat classes (Primary - 96 ha, Secondary A - 578 ha, Secondary B – 808 ha, and Secondary C – 670 ha) with associated Koala population densities (0.63/ha, 0.42/ha, 0.23/ha, and <0.1 Koalas/ha, respectively). This habitat classification was based primarily on the distribution and abundance of three preferred food tree species: Tallowwood (*Eucalyptus microcorys*), Swamp Mahogany (*E. robusta*) and Forest Red Gum (*E. tereticornis*). The estimated population densities were derived from a range of earlier studies by the authors (Phillips *et al*. 2015).

The accuracy of this information, as it applies to the study area, could not be tested. However, the information was used to estimate the carrying capacity of the habitat in the study area as suitable for approximately 556 Koalas (Phillips *et al*. 2015). These authors then considered that such a population density to be unsustainable and reduced their estimate by nearly 50% to a long-term carrying capacity of 291 Koalas (Phillips *et al*. 2015). In the PVA model, carrying capacity was set at 291 Koalas in the study area, but we need to identify the limitations that were involved with this estimate.

A total of 17 ha of good habitat for the Koala is proposed for removal during road construction in Section 10 of the highway upgrade (Table 1). This amounts to the potential loss of habitat for approximately 5 Koalas (17*0.63/2) in the study area, assuming that these animals are unable to re-establish themselves within part of their previous home-ranges. This habit loss was scheduled to occur during clearing for road construction (year 2 of the model).

Roads and Maritime Services has also committed to planting at least 130 ha of new habitat for the Koala (see section 5.3). Using similar calculations to those above, this amounts to the provision of new habitat for approximately 41 Koalas (130*0.63/2). In the PVA model, these were distributed as habitat for 25 new animals on the western side of the road and 16 new animals on the eastern side of the road (i.e. in proportion to proposed areas of planted habitat on both sides of the road). Carrying capacity was scheduled to increase to 327 (222+69-5+41) in yearly increments of three animals, beginning in year 7 (tree plantings were established in year 1), and continuing until year 15 for the western plantings and year 12 for the eastern plantings. Eucalypt plantations comprised mainly of preferred Koala food tree species and aged 6-15 years are rapidly occupied by Koalas if the animals are present nearby (Kavanagh and Stanton 2012, Rhind *et al*. 2014).

In this study, the expected loss of habitat for up to five Koalas and the proposed area of new habitat (revegetation) that will be provided for approximately 41 Koalas were treated in the model as initially reducing (for five years post road construction) then increasing (from seven to 15 years post-plantation establishment) the carrying capacity of the habitat in the study area.

Catastrophic events

Three drought years were identified over the previous 14 years (i.e. frequency of ~ 0.21), but these drought effects were considered by Phillips *et al*. (2015) as likely to affect only ridgeline areas of Koala habitat (i.e. 700/2152 ha) or 32% of Koala habitat in the study area. Estimates of a reduction in breeding success and survival due to drought in 32% of Koala habitat available were 0.85 and 1.0, respectively, of the baseline inputs for these parameters (Phillips *et al*. 2015). Hence, to calculate the average effect of drought across the entire study area (as required for the PVA), there would be no change in breeding success across 68% of the study area (1 x 0.68) but a 0.85 (or 15%) reduction within 32% of the study area (0.85 x 0.32), which, when summed, provided an overall input value of 0.952 (or 4.8%) reduction. There was no predicted reduction in animal survival in areas affected by drought (i.e. no animals died), so the input value for an average reduction in survival due to drought remains as 1.0 (i.e. no change in the parameter estimates).

The probability of a catastrophic fire event was estimated to be once in every 35 years (i.e. at a frequency of ~ 0.03), but this event was considered likely to encompass only 10% of the study area (Phillips *et al*. 2015). Estimates of a reduction in breeding success and survival due to a fire event within 10% of the Koala habitat available were 0.85 and 0.60, respectively, of the baseline inputs for these parameters (Phillips *et* *al*. 2015). So, to calculate the effect of a fire event on average breeding success across the entire study area (as required for the PVA), there would be no change in breeding success across 90% of the study area (1 x 0.9) but a 0.85 (or 15%) reduction within 10% of the study area (0.85 x 0.1), providing an overall input value of 0.985 (or 1.5%) reduction. Similarly, the input value for an average reduction in survival due to a fire event was no change across 90% of the study area (1 x 0.9) but a 0.6 (or 40%) reduction within 10% of the study area (0.60 x 0.1), providing an overall input value of 0.96 (or 4%) reduction.

5.3 Genetics studies

Two studies were conducted to profile the genetic structure and composition of the Ballina Koala population (Neaves *et al*. 2015, Norman *et al*. 2015; Appendices 3 and 2, respectively). These studies measured the levels of genetic diversity in the study area compared to surrounding areas. The Australian Museum study (Neaves *et al*. 2015) obtained tissue samples for 38 Koalas in the study area and compared these using microsatellites with 231 Koala samples from an existing database (Australian Centre for Wildlife Genomics) for four surrounding locations in NSW and south-east Queensland (Port Macquarie, Coffs Harbour, Tyagarah and Coomera). Mitochondrial DNA analyses were also performed for a total of 454 Koalas across the species' distribution to place the population in the study area within a broader context. The Southern Cross University study (Norman *et al*. 2015) analysed tissue samples for 47 Koalas collected within the study area and compared these to samples from 88 Koalas collected from nearby but outside of the study area (42 from west of the study area and south of Lismore, 30 to the north-east of Lismore and 16 from between Lismore and Casino).

Both studies reported that the levels of genetic diversity present within the Ballina Koala population (i.e. within the study area) were comparable to that found at other locations in the region. The levels of genetic variation are within the range reported for populations in northern NSW, central NSW and south-east Queensland but exceed those reported for populations in Victoria. The Australian Museum (AM) study reported no evidence of genetic structuring within the study area, but the Southern Cross University (SCU) study reported that there was genetic differentiation between the northern and southern sub-populations within the study area. This difference was explained by the northern sub-population receiving more immigrants from surrounding areas compared to the southern sub-population which is surrounded on two sides by natural barriers to Koala movement (i.e. the Richmond River and the Tuckean Broadwater). For the purposes of PVA modelling, no genetic sub-structuring was assumed for the population in the study area.

In a regional context, there was evidence for gene flow across the populations sampled in the region, but with some genetic differentiation associated with geographic distance. Both studies found evidence of long-range (up to 20 km) dispersal, although distances of up to 3.5 km were more typical, and animals that were geographically closer to each other were more likely to be closely related. Both studies provided estimates of dispersal (i.e. number of Koalas per generation), both between the study area and surrounding areas and within the study area between areas east and west of the proposed highway. These estimates assumed that dispersal was symmetrical because in most cases it was not possible to determine the direction of dispersal.

Both studies reported that the average level of inbreeding is negligible in the study area. The SCU study provided estimates of the effective number of alleles in the population in the study area, and the AM study provided frequencies for each allele in the population. This information was compiled into a spreadsheet by Dr Catherine Grueber (Sydney University) and used in the PVA modelling to obtain an estimate of the genetic diversity (number of alleles remaining) resulting from each set of scenarios.

5.4 Estimating dispersal

Dispersal occurs at multiple levels, and rates, throughout the study area and it is difficult to estimate these values. Within the study area, one aspect of dispersal (a) is an estimate of the number of animals, per generation, that have successfully contributed to the breeding pool in an adjacent sub-population. This information has been provided by the two studies of genetic diversity in the Ballina Koala population (Neaves *et al*. 2015, Norman *et al*. 2015; Appendices 3 and 2, respectively). These studies estimated that 2.9, or 5 (± 2.2), individuals (mean 3.95), respectively, move from their natal home ranges to their new breeding home ranges across a nominal line coinciding with the location of the proposed road in the study area. These data are based on a Koala generation length of 6.02 years, which was estimated for a free-ranging, *Chlamydia*-positive population in north-eastern New South Wales (Phillips 2000).

Another aspect of dispersal (b) is that which includes all movements that may occur within the Koala population, but which do not necessarily contribute to the breeding pool in an adjacent sub-population (e.g. transient animals, and animals which breed only in one sub-population but whose home-ranges are large enough to overlap parts of two sub-populations). In most years, this form of dispersal is likely to be inconsequential, as the effective rates of dispersal have already been encompassed within estimates for dispersal type (a). However, dispersal type (b) is likely to be density-dependent and so could become very important in certain years through the provision of "population rescue" when a sub-population has been severely depleted by some catastrophe, or if there is a steady decline in the size of an adjacent sub-population. Dispersal type (b) is difficult to parameterise in the model because these population density-dependent relationships are unknown. The relevance of considering this form of dispersal is that, in some years, the rate could be much greater than that normally occurring and the capacity to accommodate this may be affected by the number of connectivity structures provided.

A range of plausible estimates for dispersal were used in the PVA models, beginning with the estimates provided by the two genetic studies (i.e. mean 1.98 animals each way per year). However, because less than half (44.83%) of the females of breeding age actually bred during the year of the local field study (2014-2015), it could be argued that the number of Koala movements through the connectivity structures (if they were present) might be at least twice the numbers for dispersal estimated by the two genetics studies (i.e. more than 4 animals each way per year). Further guidance may be provided by long-term Koala radio-tracking studies elsewhere. For example, 40 (23 males and 17 females) of 195 (20.5%) radio-collared Koalas dispersed in south-east Queensland (Dique *et al*. 2003). Ninety-three percent of these dispersing animals were 20-36 months of age, with the mean straight-line distance between natal and subsequent breeding home ranges measured at 3.5 km for males (range 1.1-9.7 km) and 3.4 km for females (range 0.3-10.6 km). In the Pilliga forests of northern NSW, 6 of 32 (18.8%) radio-collared Koalas were initially captured at one location but moved to establish a new home-range during the 12 month study (Kavanagh *et al.* 2007). These 6 animals were all 2-3 years old (four males and two females). The mean daily (straight-line) movements for all animals in the study was 89 m, but this included the large daily movements (up to 897 m) when young animals were dispersing.

In the PVA modelling, only animals aged 1-4 years (both genders) were permitted to disperse. Dispersal inputs ranged from 1.98 individuals (based on the mean estimate provided by the genetics studies), through to 4, 8, 10 and 20 individuals moving each way per year to encompass a range of potential dispersal scenarios. Dispersal rates were treated as "symmetric" between the two sub-populations, as per the assumptions of the genetics analyses. Dispersal was also modelled as the number of animals dispersing rather than as a percentage of each sub-population because the smaller, eastern sub-population acted as a sink when equal percentages of each sub-population were permitted to disperse. Mortality was assumed to be zero for all dispersing animals.

5.5 Estimating immigration and emigration

The study area boundary (Figure 1) and the surrounding vegetation types were shown by the genetics studies not to constitute a barrier to Koala movements. Accordingly, the “harvest” and “supplementation” features of Vortex were used to account for emigration out of, and immigration into, the study area each year, respectively. As in the previous section (5.4), rates of immigration and emigration can be viewed as dispersal type (a) and dispersal type (b), although it is more difficult to estimate values for these parameters (especially for dispersal type b) because of the large area and perimeter involved. Fortunately, the two recent studies of genetic diversity in the Ballina Koala population (Neaves *et al*. 2015, Norman *et al*. 2015) also compared their results with other Koala populations nearby (Tyagarah and Lismore, respectively).

These studies estimated that 5.7 (± 2.8) Koalas move per year between the study area and the broader Lismore area (Norman *et al*. 2015; Appendix 2), and that 0.8 individuals move per year between the study area and the more distant Tyagarah Koala population (Neaves *et al*. 2015; Appendix 3). Given that the Lismore samples were in closer proximity to the study area (approximately 15 km vs 40 km away), we decided to model immigration and emigration in Vortex using the values of 2.85 and 2.85, respectively (sum=5.7). No estimates of dispersal type (b) could be determined for immigration and emigration, and so only the above values (2.85) were used in modelling immigration and emigration.

5.6 Mitigation options

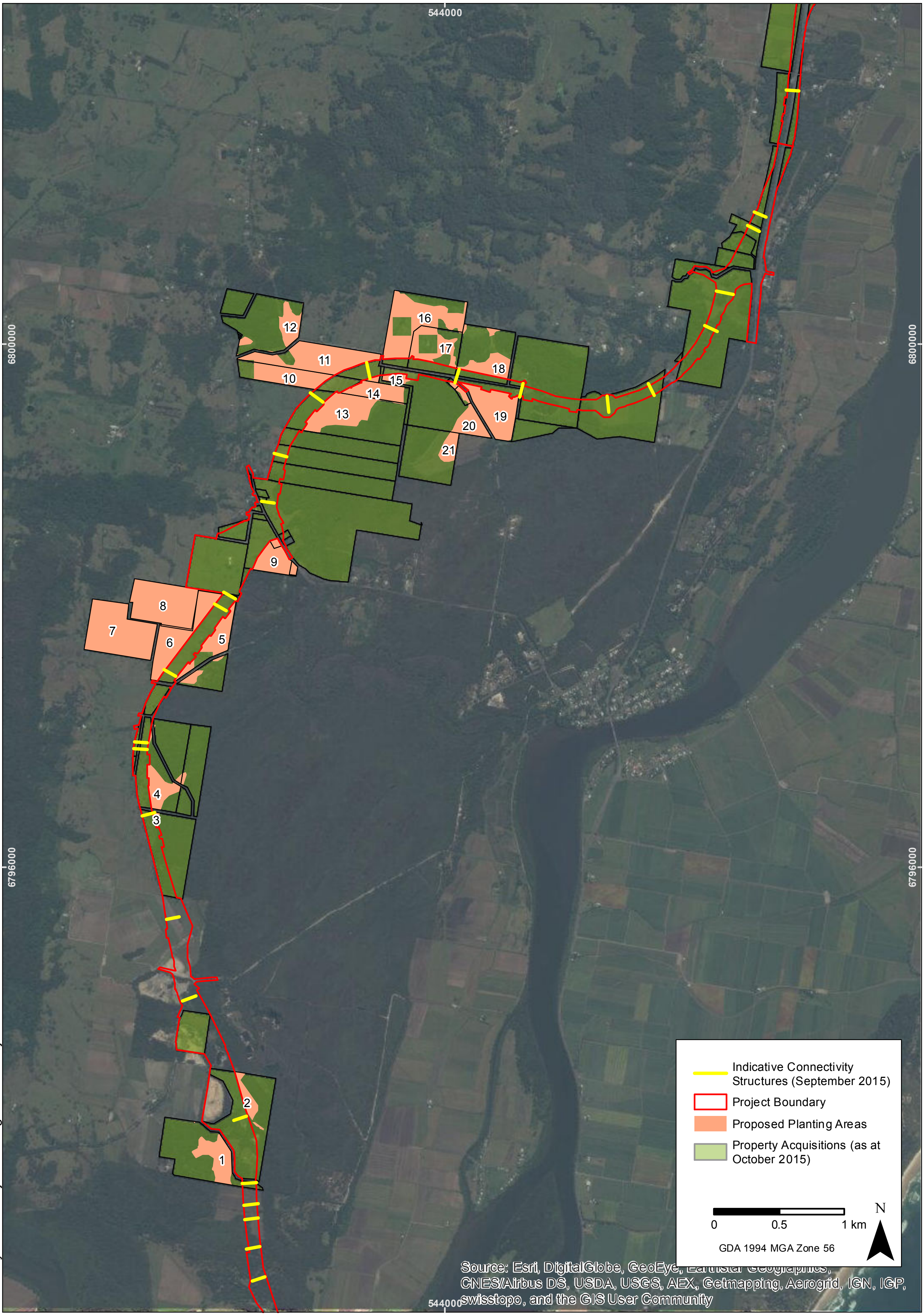
Roads and Maritime Services, in consultation with other agencies and Koala experts, have agreed to build 26 connectivity structures along the 13.5 km of Section 10 of the proposed highway upgrade, all of which are likely to have value in enhancing the dispersal and movements of Koalas in the study area (Figure 4; Appendix 4). This is a significant increase in the numbers and type (i.e. Koala-friendly designs) of these structures compared to those proposed in the EIS/SPIR (2012/2013) (Figure 5; Appendix 4).

On average, it is proposed that there will be approximately 1.9 connectivity structures per km of new road, or about one connectivity structure per 520 m of the road. With Koala home-ranges averaging about 15 ha (i.e. 437 m diameter; Kavanagh *et al.* 2007), this represents nearly one connectivity structure per Koala home-range either side of the proposed highway upgrade in Section 10, providing opportunities for most animals living near the proposed road to cross safely or to disperse.

The highway upgrade along Section 10 will also be fully-fenced, using Koala-proof floppy-top fencing, with the provision of grids near the intersections with other roads, to create a fully closed system to prevent vehicle-strike to Koalas (Appendix 4).

Roads and Maritime Services has acquired at least 621 ha (as at October 2015) of forested and cleared land near the proposed highway upgrade in Section 10, of which 151 ha is available for revegetation. Eucalypt plantations comprised mainly of preferred Koala food tree species are rapidly occupied by Koalas if the animals are present nearby (Kavanagh and Stanton 2012, Rhind *et al*. 2014). The NSW Minister for Roads and Freight has made a commitment to plant at least 130 ha of Koala food trees in the study area, if the proposed upgrade is approved, and a Koala revegetation strategy has been developed (Kavanagh and McLean 2015; Figure 4). This is equivalent to the provision of new habitat for approximately 41 Koalas (see section 5.2). As described in the previous section, this new habitat was incorporated in the model through incremental changes (from years 7-15) in the carrying capacity of the study area.

The proposed new Koala food tree plantations will also enhance dispersal between the western and the eastern sub-populations by focussing Koala movements towards the connectivity structures that will be provided, and will facilitate Koala movements through adjacent areas that are currently cleared farmland.



Drawn by: GT Project Manager: RK Project Number: 2266 Date: 02/10/2015

Section 10 - RMS Proposed Planting Areas

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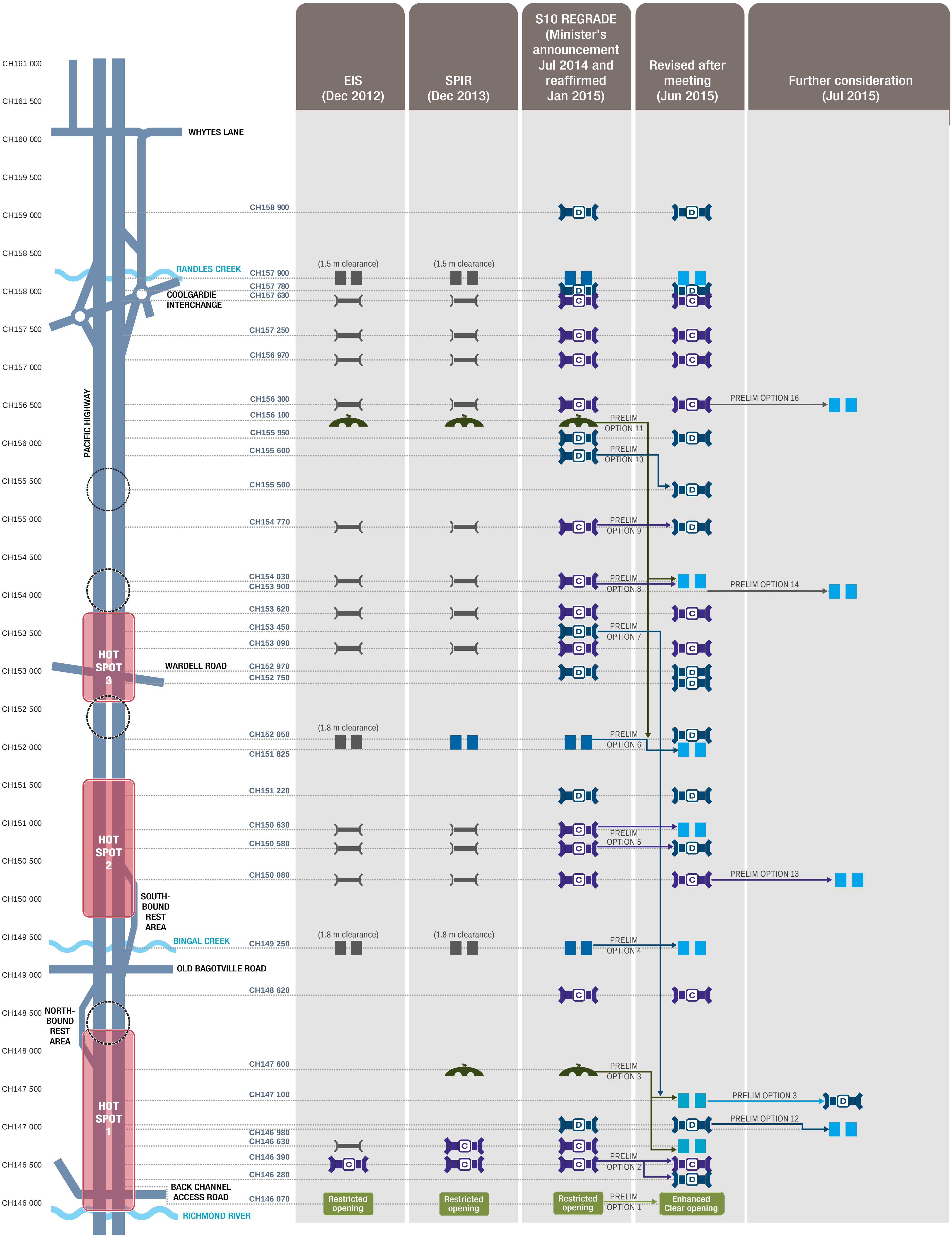
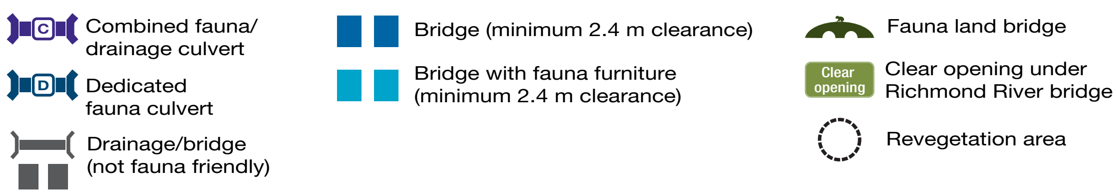
FIGURE 4

Path: T:\spatial\projects\a2200\a2266_W2B_KPoM\Maps\report\2266_Figure_4_planting.mxd

KOALA CONNECTIVITY DEVELOPMENT





Note:
(1) Koala friendly crossing shown have a minimum 2.4 m clearance

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5.7 Road impacts

Road impacts may adversely affect Koala populations through habitat fragmentation and barrier effects, as well as through direct mortality from vehicle strikes. Independent studies have shown that roadkills can be a major source of mortality for Australian wildlife, including Koalas (Taylor and Goldingay 2003, 2004, 2010; Hobday and Minstrell 2008; AMBS 2011). Over a four-year period from 2005-2008, a total of 530 Koalas was presented to veterinary clinics near Port Stephens in NSW, 205 (38.7%) of which had been struck by motor vehicles (D. Hudson, personal communication).

In the Ballina study area, of 109 "call-outs" reported by Lismore Friends of the Koala (data from 1989-2014), 35 (32.2%) Koala deaths were due to vehicle strikes, compared to 22 (20.2%) due to dog attacks, 40 (36.7%) due to disease, and 12 (11.0%) due to natural causes (Phillips *et al*. 2015). These authors reported four locations in the study area as known "hot spots" for Koala mortalities (Pacific Highway, Bruxner Highway, Wardell Road and Bagotville Road; Appendix 5), and observed six road-killed Koalas during their six-month field study (Phillips *et al*. 2015). A recent update reported that at least 10 Koalas were hit by vehicles in the study area during 2015, eight of them within the four hot spots identified (S. Phillips, *pers. comm.* 14/12/2015) (see also section 6.4).

5.8 Koala use of connectivity structures

Crossing structures (underpasses and overpasses) have been shown to be effective for Koalas provided they are large enough in cross-section, not too long (<50 m), and are combined with Koala-proof fencing and revegetation (Taylor and Goldingay 2003, AMBS 2011, RMS unpublished data). However, research is lacking on the extent to which mitigation measures reduce the risk of local extinction, given the overall context of the major linear infrastructure (Taylor and Goldingay 2010, Van der Ree *et al.* 2011). No relationship has yet been established between the numbers of connectivity structures available and the probability that an animal will cross, or the numbers of individual Koalas using them. As indicated above (section 5.4), the number of connectivity structures normally required to satisfy Koala dispersal type (a), may be less than the numbers required to facilitate Koala dispersal type (b) during the period following a catastrophe when sub-population rescue is needed. Accordingly, we modelled dispersal across a continuum of values (0.792, 1.98, 4, 8, 10, 20 animals each way per year) to investigate this possibility. In this PVA, we assumed that all 26 connectivity structures proposed for Section 10 were required to meet the needs of dispersal at each level (1.98, 10 or 20 animals), and that the worst-case scenario for road impact was that connectivity may be reduced to 40% of these levels (0.792, 4 or 8 animals). This worst-case estimate of 40% dispersal was based on each connectivity structure having a "catchment area" of 200 m (i.e. 100 m fencing either side of each connectivity structure), amounting to approximately 40% of the total length of the road.

5.9 Output measures

All PVA scenarios were modelled across a timeframe of 50 years (see section 1.2).

Each model-run in Vortex produced comprehensive output for each sub-population (east and west of the proposed highway upgrade) and for the overall population. These outputs included tables showing the projected number of Koalas remaining after 50 years, the probability of extinction after 50 years, the population growth rate, the number of alleles remaining in the population, and the variability around each of these estimates based on 1000 simulations of each modelled scenario. A wide range of graphical outputs can also be displayed.

6. Modelled Scenarios

6.1 Impact assessment

The impact of the proposed road was assessed by comparing population projections based on differences in the rate of dispersal between two sub-populations as influenced by the proposed connectivity structures. The provision of supplementary habitat for Koalas in the study area was modelled through an increase in the projected carrying capacity of the habitat. Management options were investigated by varying the levels of key population parameters (e.g. fecundity, mortality) in a series of sensitivity tests.

Before any impacts due to the road could be assessed, it was important to untangle any confounding effects that may be caused by splitting the population into two sub-populations. That is, smaller populations are inherently more prone to extinction than larger populations, regardless of any road effects. Accordingly, all modelled scenarios were conducted on the basis of comparisons between two sub-populations in which the level of dispersal was either unconstrained (i.e. the no-road scenario) or constrained (i.e. the presence of the road). For the purposes of assessing the impact of the proposed road, it was assumed (expert workshop discussions, 14 October 2015 organised by NSW Koala Expert Advisory Committee) that the 26 connectivity structures to be provided (section 5.6) would either cater fully for the dispersal needs of the population (i.e. 100% connectivity) – and therefore result in no impact of the road – or, as a worst case scenario, would limit the rate of dispersal to 40% of the numbers of animals attempting to disperse. The worst-case estimate of 40% dispersal was based on each connectivity structure having a “catchment area” of 200 m (i.e. 100 m fencing either side of each connectivity structure), amounting to approximately 40% of the total length of the road. It was also assumed that the proposed road would be fully fenced to prevent any additional Koala mortalities. The “worst-case” impact of the road could therefore be estimated as a percentage by dividing the projected number of animals remaining in the population after 50 years in the 40% connectivity scenario by the numbers remaining after 50 years in the 100% connectivity scenario, and subtracting the result from 100.

The scenarios modelled included the following:

- No habitat supplementation or management interventions, but comparing dispersal rates either unconstrained (100% connectivity) or constrained (40% connectivity) – including sensitivity tests to determine the effects of uncertainty in the estimates of demographic parameters. Note, this scenario is simply provided as a reference point because road construction will require the removal of habitat for some animals.

- No habitat supplementation or management interventions, but comparing dispersal rates either unconstrained (100% connectivity) or constrained (40% connectivity), including the loss of habitat for 5 Koalas during road construction. These scenarios also included sensitivity tests to determine the effects of uncertainty in the estimates of demographic parameters.

- Effect of habitat supplementation for up to 41 Koalas after accounting for the loss of habitat for 5 Koalas. These scenarios also include sensitivity tests for an overall reduction in mortality by 20% across all age-gender classes, increasing population fecundity by 20%, and both reducing mortality by 20% and increasing fecundity by 20%. Note, these variations in fecundity and mortality rate are also provided to indicate the potential for management (unspecified) to affect population outcomes.

- Effect of reducing mortality by either 4 or 8 young animals per year, combined with habitat supplementation.

In each of these scenarios, the PVA models incorporated dispersal estimates ranging from 1.98-20 animals per year moving each way across the proposed road.

6.2 Sensitivity analysis

Sensitivity testing was undertaken to achieve an understanding of the most influential variables in the analysis, and to determine the effects on model results of uncertainty in the estimates of key demographic parameters. The sensitivity test (ST) function in Vortex was used to simultaneously compare a range of inputs for certain parameters, while holding all the rest of the parameters constant. Variables tested in this way were breeding success, female and male mortality rates by age-class, initial population size, carrying capacity and the number of lethal alleles in the population. Subsequently, the effects of uncertainty in the estimates of fecundity, mortality and dispersal on model results were tested by varying these parameters up or down by 20% of their base values (Phillips *et al.* 2015). Inbreeding depression was also assumed to be present for these sensitivity tests.

6.3 Road effects models: role of habitat supplementation

Habitat supplementation for up to 41 Koalas after accounting for the loss of habitat for 5 Koalas was achieved by initially reducing the carrying capacity of the habitat in the study area by five animals for 6 years after clearing for road construction, followed by small increases in carrying capacity each year from 7-15 years after plantation establishment (see section 5.2). These scenarios also included sensitivity tests for an overall reduction in mortality by 20% across all age-gender classes, increasing population fecundity by 20%, and both reducing mortality by 20% and increasing fecundity by 20%. The primary reason for including these scenarios was to assess the effects of uncertainty in these parameter values, but they also provide an indication of the potential for management (unspecified) to affect population outcomes (e.g. by reducing Koala mortality through fencing on other local roads, controlling dog predation, and by increasing Koala fecundity by limiting the frequency and severity of disease in the population through the application of a *Chlamydia* vaccine, or both).

6.4 Road effects models: applying management to control mortality

Long-term data collected by the Lismore Friends of the Koala group for the numbers and locations of Koalas killed by vehicles on roads in the study area averaged 1.23 animals per year, although annual mortalities of 4-6 animals were considered more likely (Phillips *et al*. 2015). These authors observed six Koala mortalities caused by vehicle-strike during the six months of their field study, and at least 10 mortalities in 2015 (S. Phillips, *pers. comm*. 14/12/2015). Four main road-kill “hot-spots” were identified (Appendix 5). The same long-term data set also showed that at least 1.64 Koalas were killed annually by predation by domestic dogs. This information suggests that there may be opportunities for management to reduce the numbers of “avoidable” Koala mortalities by up to four or possibly eight animals per year in the study area. These two scenarios are included because they represent achievable objectives for management because reducing mortality by 4 or 8 animals could occur by fencing known road-kill hotspots, and by controlling local dog predation.

Reducing Koala mortality by either 4 or 8 animals in the study area due to management control was modelled by eliminating “harvest” (emigration) from the study area (i.e. 2.85 animals per year) and adding the balance (either 1.15 or 5.15 animals per year, respectively) to the existing level of 2.85 for “supplementation” (immigration). This reduction in mortality was applied at the rate of 40% for young females (1-3 years old) and 60% for young males (1-4 years old) because of the greater propensity for males to be killed by cars (Phillips *et al*. 2015).

7. Results

7.1 Deterministic population growth rate

All scenarios modelled, with or without the proposed highway upgrade, showed a gradual decline in this population of Koalas. Deterministic projections of population growth (i.e. in the absence of catastrophes and other unplanned stochastic events) were negative. This is, in 2014-2015, the birth rate was insufficient to offset the death rate in this population. The exponential rate of increase for the population was $r=-0.0049$, the annual rate of change was $\lambda=0.9951$, and the per-generation rate of change or "net replacement rate" was $R_0=0.8092$. The population was found likely to persist for at least 50 years under most scenarios, but at much reduced numbers (Figure 6; Tables 4-5).

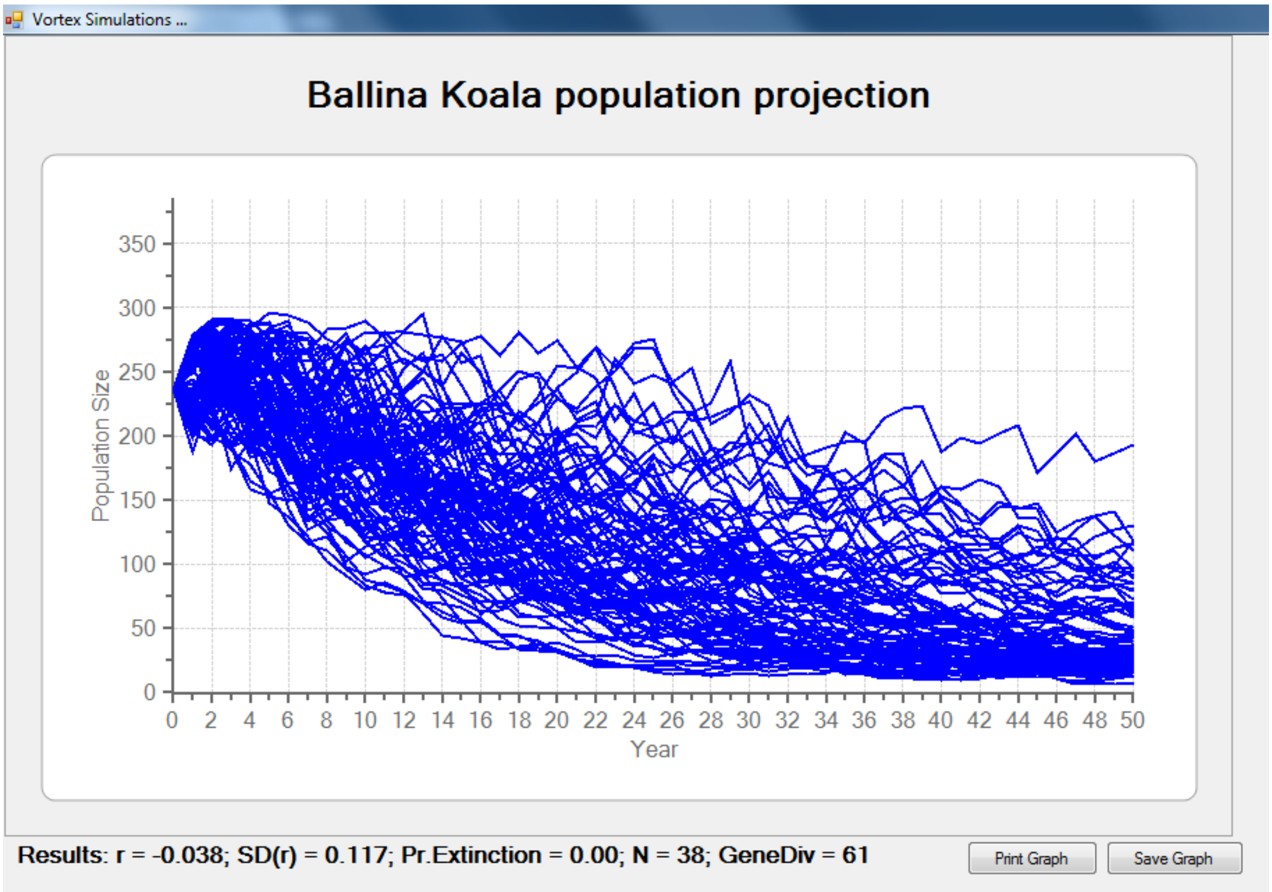


Figure 6: Projected Koala population decline in the study area over 50 years, in the absence of the proposed highway (results of 1000 simulations). The projection incorporates the likelihood of drought and fire in the study area, together with a small allowance (2.85 individuals per year) for both immigration and emigration. The projection also assumes the presence of inbreeding in the population.

Assumptions about the presence of inbreeding depression in the population had a significant effect on the results. Including inbreeding depression and the presence of lethal alleles in all models had the effect of reducing population size projections by approximately 20-40% of those estimated when inbreeding depression and the presence of lethal alleles was switched "off" in the analyses. The genetics studies by Neaves *et al*. (2015) and Norman *et al*. (2015) both reported very low levels of inbreeding in the Ballina Koala population but, to be conservative, we presented our final PVA results with the assumption that inbreeding depression was present in the population.

7.2 Sensitivity tests – identifying the most influential variables

Sensitivity tests were applied within Vortex to investigate the influence of parameter estimates for key population variables. There will always be uncertainty surrounding the results of "snapshot" estimates derived from short-term field studies because these estimates vary from one year to another but, in the absence of long-term population data, the real question is - for which variables are these errors likely to have a significant impact on the results? Conversely, which variables are most likely to influence population viability outcomes if they can be controlled or manipulated by management?

Sensitivity tests showed that breeding success (population fecundity; Figure 7) and female mortality rates for both juveniles and adults (Figure 8) were highly influential in the results of the PVA (i.e. in projected population sizes). This means that any errors in the estimation of these two variables are likely to have a significant effect on the results, and also that efforts to manipulate these variables through management are likely to have a beneficial effect on population viability. In contrast, sensitivity tests for other variables, including male mortality rates for juveniles, sub-adults and adults (Figure 9), initial population size (Figure 10), habitat carrying capacity (Figure 11), and the number of lethal alleles in the population (Figure 12), showed that management attempts to vary these parameters would be unlikely to have a large effect on the results, and, of course, that errors in the initial estimates for these parameters are unlikely to be of major concern for the analysis.

The importance of birth rates (breeding success) being adequate to cover death rates (mortality) in this population is clearly shown in Figure 10 where population size is projected to decline rapidly. However, it should be noted that the declines observed in year 1 for initial population sizes of 500 and 400 animals were due to carrying capacity remaining capped at 291 individuals in the model. Management efforts to improve breeding success and/or to reduce mortality would be highly beneficial for this population. It is likely that by reducing mortality of females in particular (Figures 8 and 9), that breeding success in the population would also increase.

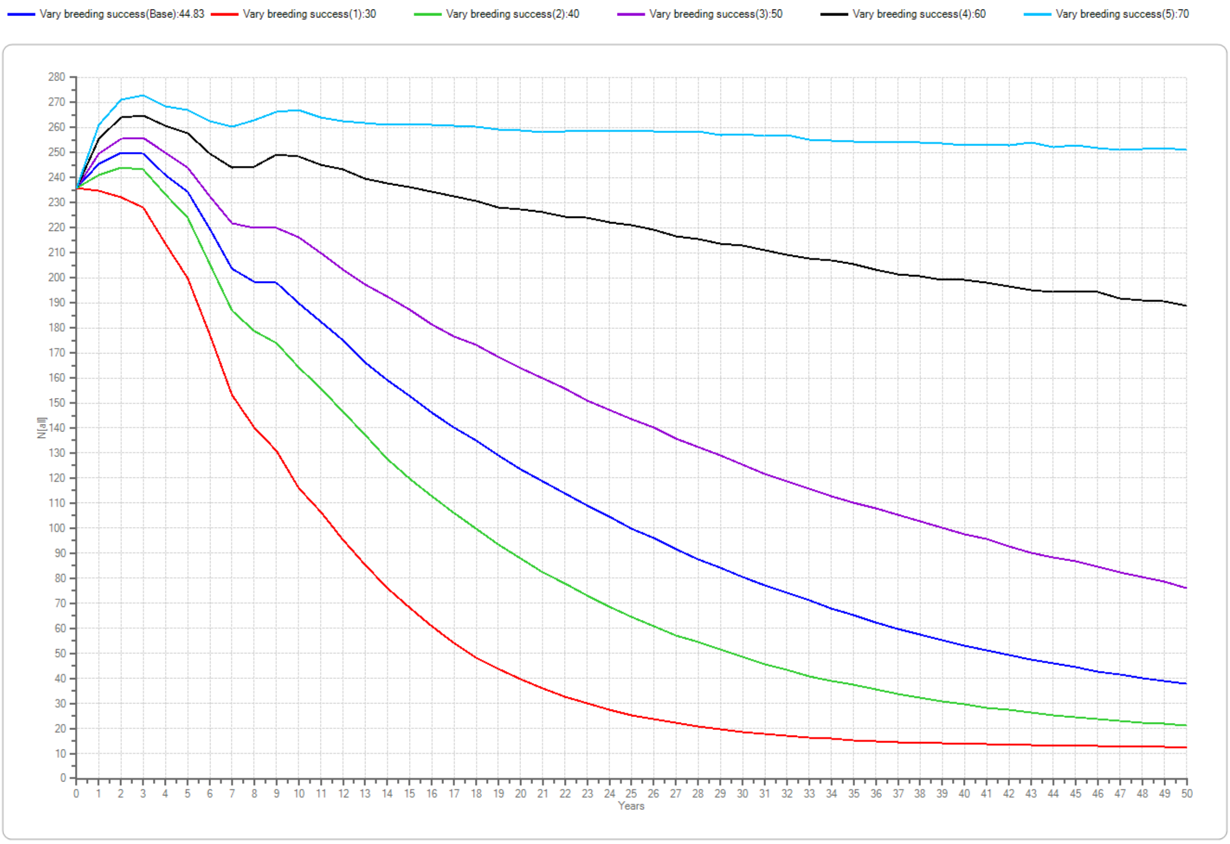
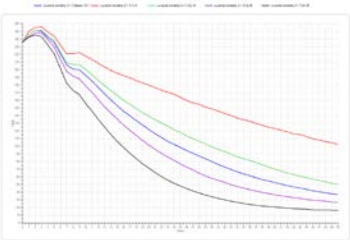
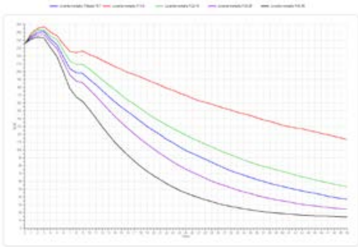


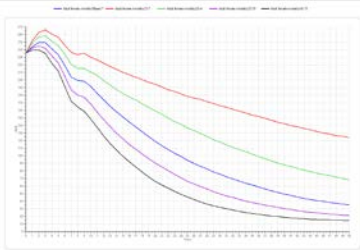
Figure 7: Sensitivity tests showing the effect of varying breeding success from 30% (red line) to 70% (turquoise line) on Koala population size after 50 years. The blue line (44.83%) shows the baseline estimate used in this study.



(a) Juvenile females (0-1 years)

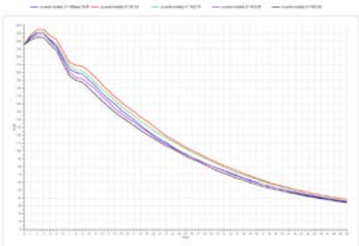


(b) Sub-adult females (1-2 years)

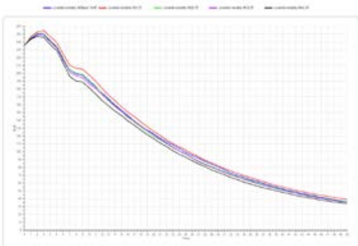


(c) Adult females (3+ years)

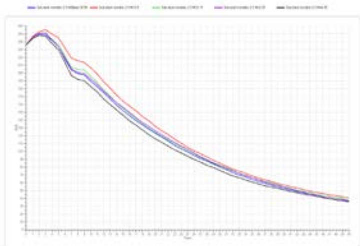
Figure 8: Sensitivity tests showing the effect of varying female mortality in age-classes 0-1 and 1-2 years from 5% (red line) to 35% (black line) on Koala population size after 50 years. For adult females, the range was from 1% (red line) to 13% (black line). In each case (a-c), the blue line shows the baseline estimates used in this study.



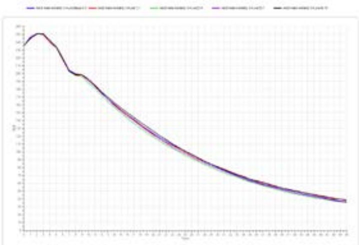
(a) Juvenile males (0-1 years)



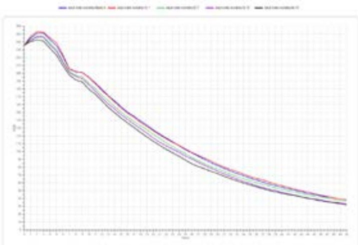
(b) Juvenile males (1-2 years)



(c) Sub-adult males (2-3 years



(d) Adult males (3-4 years)



(e) Adult males (4+ years)

Figure 9: Sensitivity tests showing the effect of varying male mortality in age-classes 0-1, 1-2, and 2-3 years from 5% (red line) to 35% (black line) on Koala population size after 50 years. For adult males, the range was from 1% (red line) to 10% (black line). In each case (a-e), the blue line shows the baseline estimates used in this study.

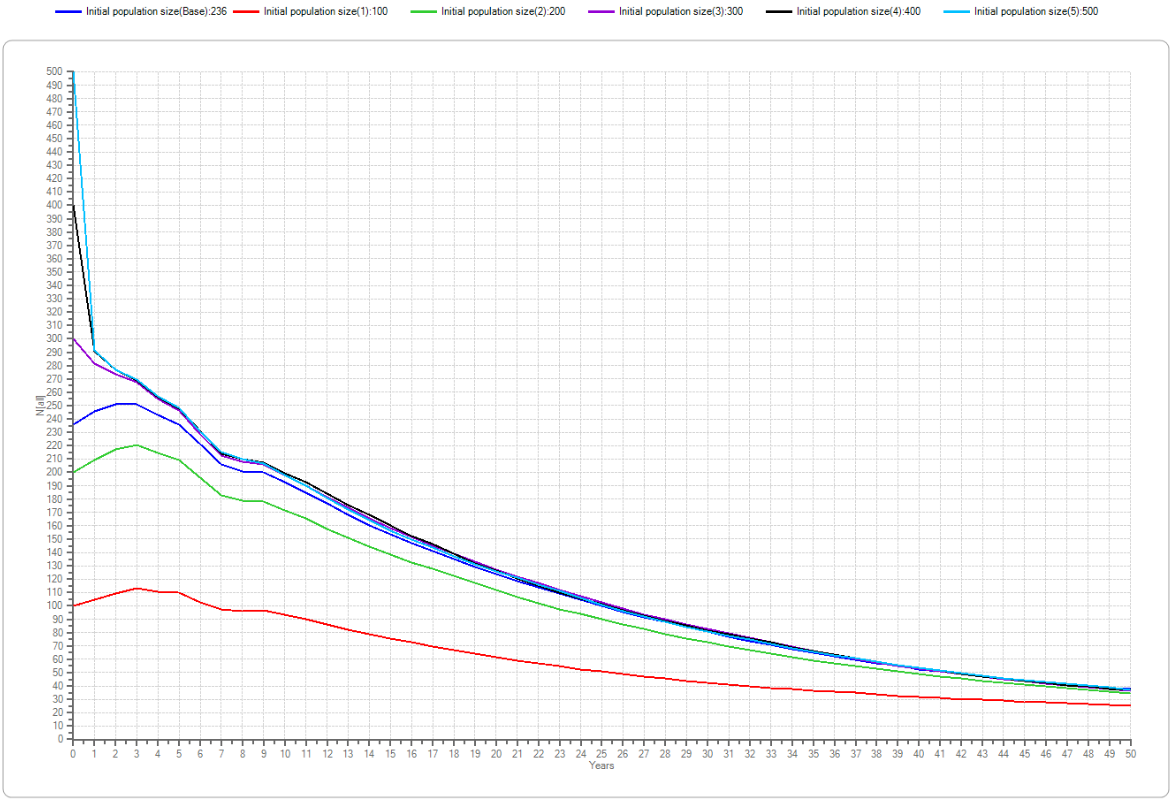


Figure 10: Sensitivity tests showing the effect of varying initial population size from 100 (red line) to 500 (turquoise line) on Koala population size after 50 years. The blue line shows the baseline estimate (236) used in this study. Note the effect of the cap on carrying capacity (291) in these simulations.

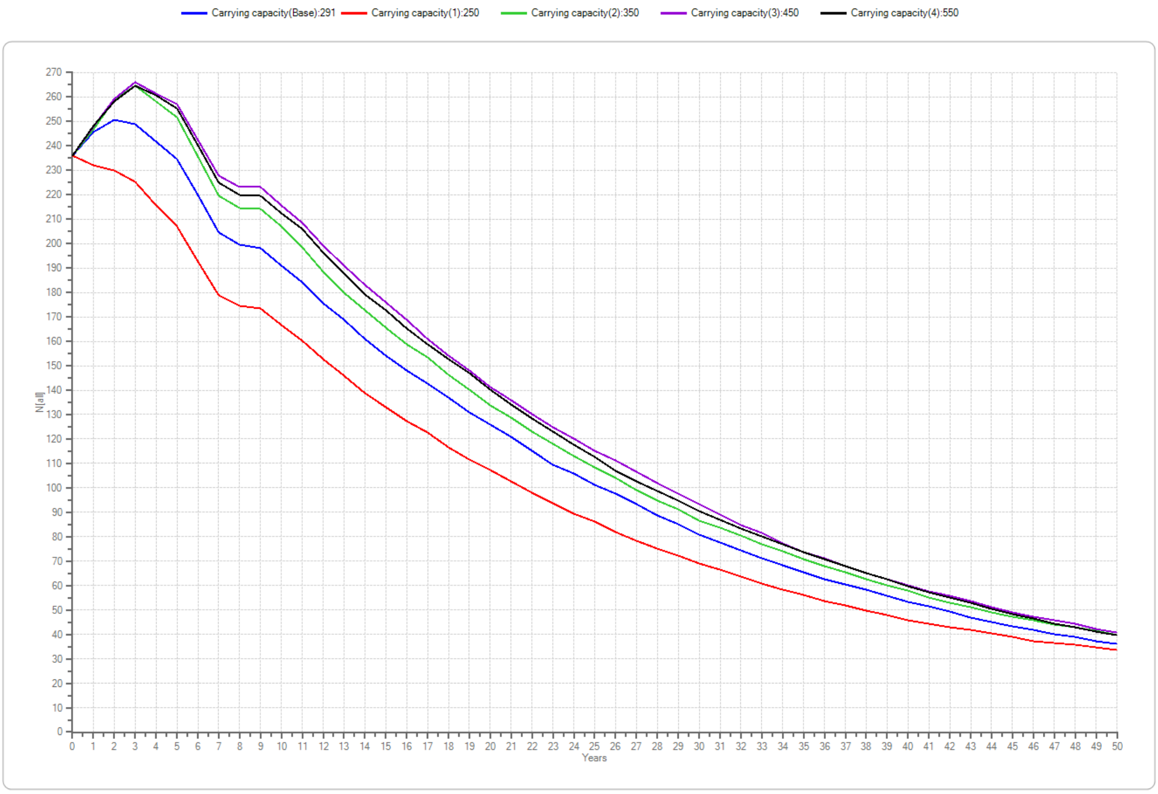


Figure 11: Sensitivity tests showing the effect of varying carrying capacity of the habitat from 250 (red line) to 550 animals (black line) on Koala population size after 50 years. The blue line shows the baseline estimate (291) used in this study.

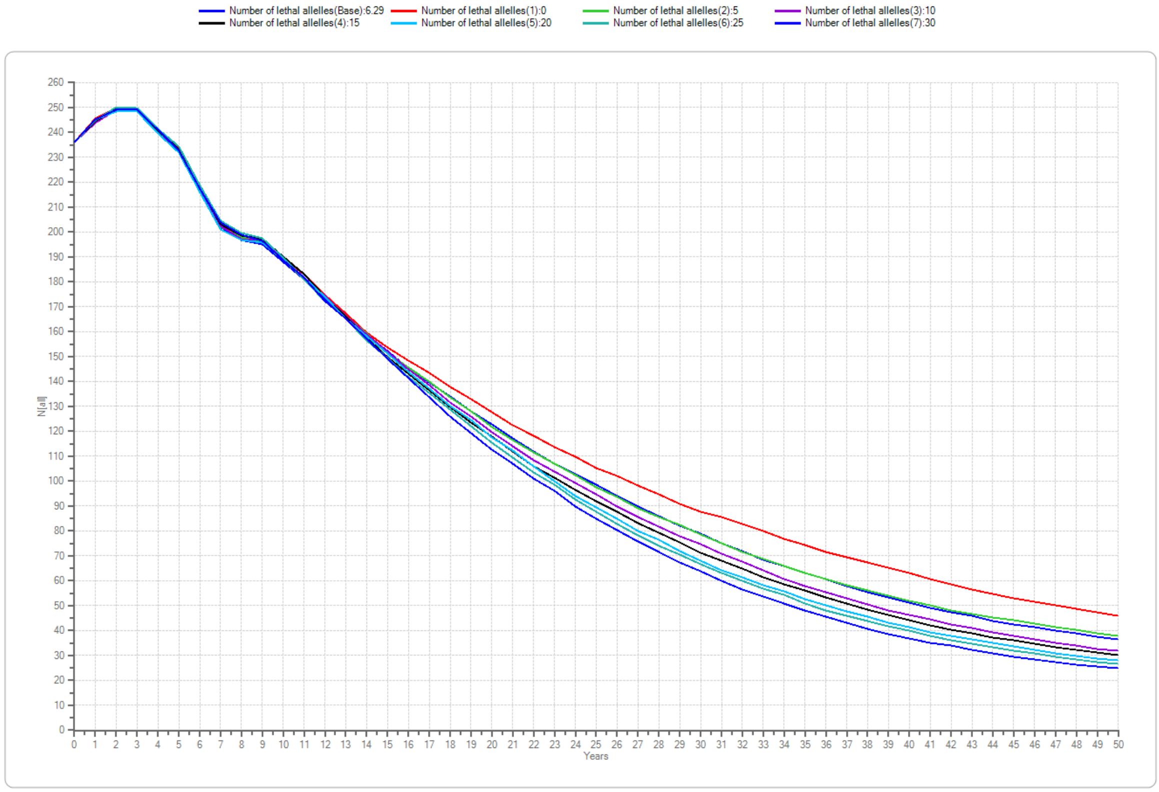


Figure 12: Sensitivity tests showing the effect of varying the number of lethal alleles in the population from 0 (red line) to 30 (lower blue line) on Koala population size after 50 years. The blue line near upper-middle shows the baseline "default" estimate (6.29) used in this study.

7.3 Effects of the proposed road – varying dispersal and connectivity

Three primary considerations need to be dealt with before the effects of the proposed road can be estimated. Firstly, it is important to untangle any confounding effects that may be caused by splitting the population into two sub-populations. This is because Vortex could calculate the probability of extinction for two sub-populations as greater than that for a single population of the same size, although this was not observed. Secondly, dispersal rates and the directions of animal movement between each sub-population are difficult to estimate, and these factors are likely to vary from one year to another depending on population density and population size. The minimum rate of dispersal in the study area was identified by the two genetics studies as approximately 3.95 animals (i.e. 1.98 each way) per year (section 5.4), although dispersal rates of up to 40 animals (i.e. 20 each way) were also modelled. Without further information, it was assumed (as did the genetics studies) that dispersal was symmetric (equal numbers of animals dispersing from west to east, and from east to west). Thirdly, relationships between dispersal rates and the numbers (or type) of connectivity structures provided are not well established. For the purposes of assessing the impact of the proposed road, it was assumed that the 26 connectivity structures to be provided (section 5.6) would either cater fully for the dispersal needs of the population (i.e. 100% connectivity) – and therefore result in no impact of the road – or, as a worst case scenario, would limit the rate of dispersal to 40% of the numbers of animals attempting to disperse. This result could also be interpreted as the likely outcome if only 40% of the planned connectivity structures were provided. The impact of the road would therefore be estimated as a percentage by dividing the projected number of animals remaining in the population after 50 years in the 40% connectivity scenario by the numbers remaining after 50 years in the 100% connectivity scenario, and subtracting the result from 100.

For analysis, the population was treated as two sub-populations divided by the location of the proposed road, but with full dispersal and connectivity between them to the extent indicated by the genetics results. A similar projected population decline was observed (Figure 13) to that shown in Figure 6.

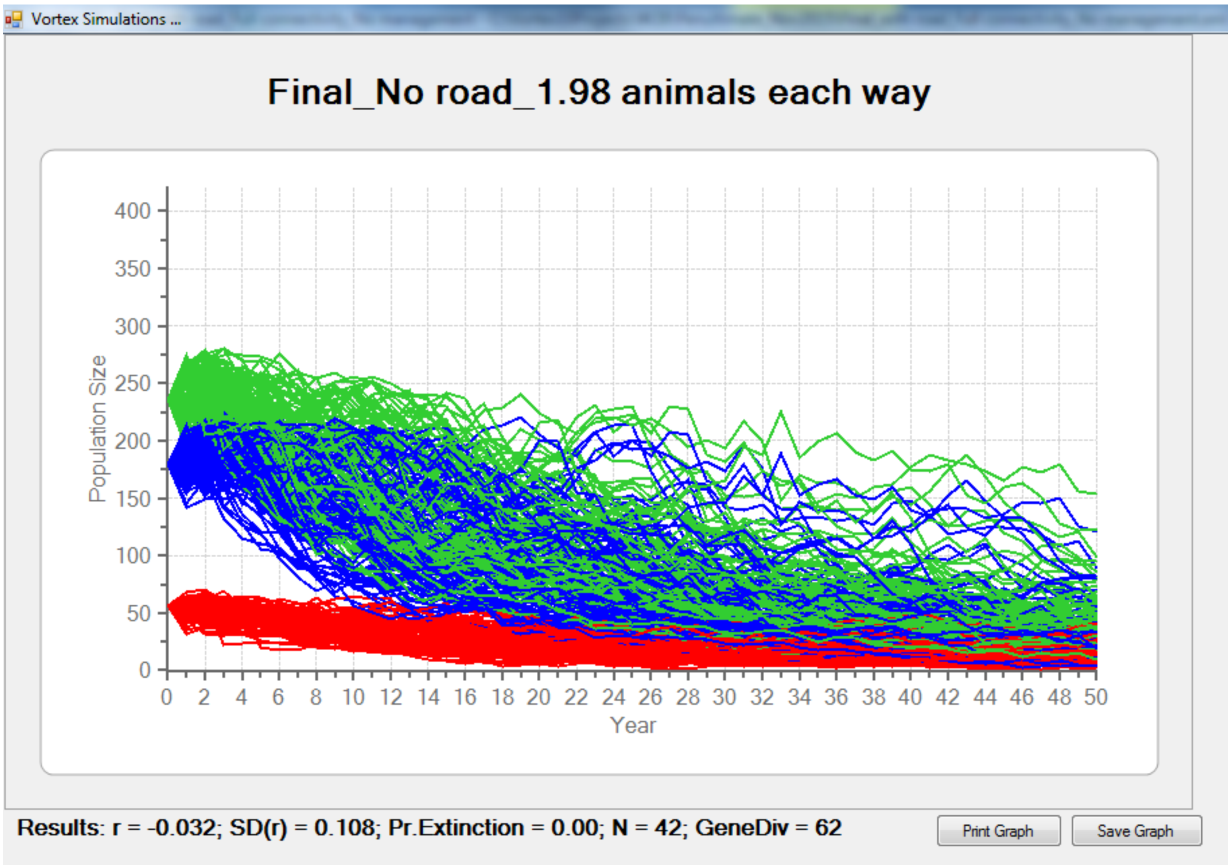


Figure 13: Projected Koala population decline over 50 years, without the proposed highway. Parameter estimates and model settings as for Figure 6, except that two sub-populations are modelled with 1.98 animals dispersing each way, per year, between them. The smaller, eastern sub-population is indicated in red, the larger western sub-population in blue, and the total population in green.

The results showed that, at the minimum levels of dispersal estimated to occur in the study area (1.98 animals moving each way per year), there was virtually no impact of the road on projected Koala population size after 50 years when reduced dispersal and connectivity was assumed (i.e. 100–[42.1/42.3*100] = -0.5%) (Table 4). If 10 animals were dispersing each way per year, there could be a 4% impact on the population if the connectivity structures limited dispersal to only 40% of this number (i.e. 100-[41.7/43.4*100] = -3.9%) (Table 4). Similarly, the impact of the road could increase to 8% if 20 animals were dispersing each way per year (i.e. 100-[43.0/46.8*100] = -8.1%) (Table 4).

Under all scenarios, with and without the road, the projected Koala population size in the study area declined substantially over the 50 year time frame (Table 4). However, varying levels of dispersal, and varying levels of connectivity, had relatively minor impacts on projected Koala population size. The final number of alleles estimated within the Koala population under each of the above scenarios ranged from 4.81-4.96.

Note, that these scenarios do not include the initial loss of habitat for 5 Koalas, or the provision of 130 ha of new Koala habitat; these are included in the following section (Section 7.4).

It should be understood that the data reported in the following series of tables are the results of simulations (i.e. probability based on 1000 runs analysed for each scenario using different values for each parameter depending on its range of variability) and so can vary slightly each time that a scenario is run using the same data inputs.

Table 4: Impacts of the proposed road on the Ballina Koala population near Wardell under a range of dispersal scenarios, not including initial habitat loss due to clearing for road construction.

All scenarios include the same demographic inputs, the same likelihood of two types of catastrophes, and 2.85 animals emigrating out of, and 2.85 animals immigrating into, the study area. Data inputs from Neaves *et al*. (2015), Norman *et al*. (2015) and Phillips *et al*. (2015). Assumes the presence of inbreeding depression and lethal alleles.

P(E), probability of extinction; N, estimated number of individuals present after 50 years.

Scenarios	Population P(E)	Population N	Western sub-Pop P(E)	Western sub-Pop N	Eastern sub-Pop P(E)	Eastern sub-Pop N
Single population	0.00	37.7	na	na	na	na
Two sub-populations, with dispersal 0 animals each way per year (zero connectivity)	0.00	38.2	0.00	23.6	0.01	14.8
Two sub-populations, with dispersal 1.98 animals each way per year (100% connectivity) **i.e. BASIC NO-ROAD SCENARIO**	0.00	42.3	0.00	27.4	0.02	15.3
Two sub-populations, with dispersal 0.792 animals each way per year (i.e. 40% connectivity)	0.00	42.1	0.00	27.0	0.01	15.4
Two sub-populations, with dispersal 10 animals each way per year (100% connectivity)	0.00	43.4	0.00	33.5	0.14	11.2
Two sub-populations, with dispersal 4 animals each way per year (i.e. 40% connectivity)	0.00	41.7	0.00	27.9	0.03	14.2
Two sub-populations, with dispersal 20 animals each way per year (100% connectivity)	0.00	46.8	0.00	41.6	0.34	7.3
Two sub-populations, with dispersal 8 animals each way per year (i.e. 40% connectivity)	0.00	43.0	0.00	31.7	0.09	12.2

7.4 Effects of the proposed road – initial habitat loss and revegetation

The provision of new habitat for up to 41 Koalas, following the loss of habitat for five animals during road construction, made little difference to the projected outcomes for the population; only fractional improvements were indicated by the modelling (Table 5). The same relativities between dispersal rates and connectivity in terms of projected population outcomes (Table 4) were observed when carrying capacity was raised to account for the planting of supplementary habitat (Table 5). This was because the deterministic decline in this Koala population ensured that there were not enough animals present to utilise the new habitat provided. The final number of alleles estimated within the Koala population under each of the above scenarios ranged from 4.86-4.94.

Table 5: Impacts of the proposed road on the Ballina Koala population near Wardell with revegetation and after accounting for initial habitat loss.

All scenarios include the same demographic inputs, the same likelihood of two types of catastrophes, 2.85 animals emigrating out of, and 2.85 animals immigrating into, the study area, and changes to habitat carrying capacity to model the effects of losing habitat for five Koalas during road construction followed by the provision of new habitat for up to 41 Koalas. Data inputs from Neaves *et al*. (2015), Norman *et al*. (2015) and Phillips *et al*. (2015). Assumes the presence of inbreeding depression and lethal alleles.

P(E), probability of extinction; N, estimated number of individuals present after 50 years.

Scenarios	Population P(E)	Population N	Western sub-Pop P(E)	Western sub-Pop N	Eastern sub-Pop P(E)	Eastern sub-Pop N
Two sub-populations, with revegetation: dispersal 0 animals each way per year (zero connectivity)	0.00	39.4	0.00	24.8	0.02	14.8
Two sub-populations, with revegetation: dispersal 1.98 animals each way per year (100% connectivity) **<u>i.e. WITH-ROAD SCENARIO, assuming no loss of connectivity</u>**	0.00	42.5	0.01	27.3	0.02	15.5
Two sub-populations, with revegetation: dispersal 0.792 animals each way per year (i.e. 40% connectivity) **<u>i.e. WITH-ROAD SCENARIO, assuming reduced connectivity</u>**	0.00	42.0	0.01	26.8	0.01	15.5
Two sub-populations, with revegetation: dispersal 10 animals each way per year (100% connectivity)	0.00	43.8	0.00	33.7	0.14	11.4

Two sub-populations, with revegetation: dispersal 4 animals each way per year (i.e. 40% connectivity)	0.00	42.5	0.00	27.4	0.02	15.4
Two sub-populations, with revegetation: dispersal 20 animals each way per year (100% connectivity)	0.00	47.8	0.00	42.5	0.34	7.4
Two sub-populations, with revegetation: dispersal 8 animals each way per year (i.e. 40% connectivity)	0.00	43.5	0.00	31.9	0.08	12.4

7.5 Effects of the proposed road - mortality reduced by 20 percent

A reduction in mortality by 20% across all age-gender classes each year would have a marked effect in improving Koala population viability (Table 6). If this reduced level of mortality could be achieved through local management efforts (e.g. comprehensive fencing arrangements on other roads in the study area), the Ballina Koala population is likely to remain viable in the long term, whether the highway upgrade is present or not. The range of estimates modelled for dispersal showed this variable to have only modest effects (<10%) on projected population size (Table 6). The final number of alleles estimated within the Koala population ranged from 5.14-5.18 for the models presented in Table 6.

The exponential rate of increase for the population when mortality is reduced each year by 20% was r=0.0220, the annual rate of change was λ=1.0223, and the per-generation rate of change or "net replacement rate" was R_0=0.9277.

Table 6: Impacts of the proposed road on the Ballina Koala population near Wardell when mortality is reduced by 20% across all age-gender classes.

All scenarios include the same demographic inputs, the same likelihood of two types of catastrophes, 2.85 animals emigrating out of, and 2.85 animals immigrating into, the study area, and changes to habitat carrying capacity to model the effects of losing habitat for five Koalas during road construction followed by the provision of new habitat for up to 41 Koalas. Data inputs from Neaves *et al*. (2015), Norman *et al*. (2015) and Phillips *et al*. (2015). Assumes the presence of inbreeding depression and lethal alleles.

P(E), probability of extinction; N, estimated number of individuals present after 50 years.

Scenarios	Population P(E)	Population N	Western sub-Pop P(E)	Western sub-Pop N	Eastern sub-Pop P(E)	Eastern sub-Pop N
Two sub-populations, with revegetation: dispersal 1.98 animals each way per year, and mortality reduced by 20%	0.00	90.8	0.00	66.2	0.00	24.7
Two sub-populations, with revegetation: dispersal 4 animals each way per year, and mortality reduced by 20%	0.00	97.4	0.00	72.5	0.01	25.0
Two sub-populations, with revegetation: dispersal 10 animals each way per year, and mortality reduced by 20%	0.00	98.5	0.00	75.2	0.02	23.8
Two sub-populations, with revegetation: dispersal 20 animals each way per year, and mortality reduced by 20%	0.00	100.2	0.00	81.2	0.07	20.4

7.6 Effects of the proposed road – fecundity increased by 20 percent

A 20% increase in fecundity of breeding-age females would significantly improve the long-term prospects for this population (Table 7). Increasing population fecundity by 20% would have a greater effect (almost 20%) on population viability than reducing population mortality by 20%. If this increase in fecundity could be achieved through local management efforts (e.g. in part by reducing mortality and thereby increasing the numbers of breeding females, or by vaccinating a proportion of the population for *Chlamydia*), the Koala population is likely to remain viable under all scenarios, whether the highway upgrade is present or not. The range of estimates modelled for dispersal showed this variable to have only modest effects (~<1%) on projected population size (Table 7). The final number of alleles estimated within the Koala population ranged from 5.15-5.18 for the models presented in Table 7.

The exponential rate of increase for the population when fecundity is increased each year by 20% was r=0.0287, the annual rate of change was λ=1.0291, and the per-generation rate of change or "net replacement rate" was R_0=0.9711.

Table 7: Impacts of the proposed road on the Ballina Koala population near Wardell when fecundity is increased by 20%.

All scenarios include the same demographic inputs, the same likelihood of two types of catastrophes, 2.85 animals emigrating out of, and 2.85 animals immigrating into, the study area, and changes to habitat carrying capacity to model the effects of losing habitat for five Koalas during road construction followed by the provision of new habitat for up to 41 Koalas. Data inputs from Neaves *et al*. (2015), Norman *et al*. (2015) and Phillips *et al*. (2015). Assumes the presence of inbreeding depression and lethal alleles.

P(E), probability of extinction; N, estimated number of individuals present after 50 years.

Scenarios	Population P(E)	Population N	Western sub-Pop P(E)	Western sub-Pop N	Eastern sub-Pop P(E)	Eastern sub-Pop N
Two sub-populations, with revegetation: dispersal 1.98 animals each way per year, and fecundity increased by 20%	0.00	112.8	0.00	87.0	0.01	25.9
Two sub-populations, with revegetation: dispersal 4 animals each way per year, and fecundity increased by 20%	0.00	112.8	0.00	85.3	0.01	27.6
Two sub-populations, with revegetation: dispersal 10 animals each way per year, and fecundity increased by 20%	0.00	113.0	0.00	87.8	0.01	25.5
Two sub-populations, with revegetation: dispersal 20 animals each way per year, and fecundity increased by 20%	0.00	114.5	0.00	91.9	0.06	23.8

7.7 Effects of the proposed road – increasing fecundity by 20% and reducing mortality by 20%

A 20% increase in annual fecundity of breeding-age females, combined with an overall 20% reduction in mortality across all age-gender classes each year, was projected to have a major beneficial effect on long-term viability of this Koala population (Table 8). Indeed, population projections showed that, if these two actions could be achieved, the combined effects of these two actions would result in population increases of approximately 186% and 231%, respectively, above those projected to result from either increasing fecundity or reducing mortality alone (Table 8). The projected population gains were also nearly five times greater (494%) than scenarios where neither fecundity nor mortality was manipulated (Tables 4, 5 and 8).

The deterministic rate of growth for the Ballina Koala population would be firmly in the positive if both management objectives (increasing fecundity and reducing mortality) could be achieved. In this scenario, the exponential rate of increase for the population was $r=0.0561$, the annual rate of change was $\lambda=1.0577$, and the per-generation rate of change or "net replacement rate" was $R_0=1.1133$.

The range of estimates modelled for dispersal again showed this variable to have little influence in the outcomes of the population projections (Table 8). The final number of alleles estimated within the Koala population ranged from 5.34-5.35 for the models presented in Table 8.

Table 8: Impacts of the proposed road on the Ballina Koala population near Wardell when fecundity is increased by 20% and mortality is reduced by 20%.

All scenarios include the same demographic inputs, the same likelihood of two types of catastrophes, 2.85 animals emigrating out of, and 2.85 animals immigrating into, the study area, and changes to habitat carrying capacity to model the effects of losing habitat for five Koalas during road construction followed by the provision of new habitat for up to 41 Koalas. Data inputs from Neaves *et al*. (2015), Norman *et al*. (2015) and Phillips *et al*. (2015). Assumes the presence of inbreeding depression and lethal alleles.

P(E), probability of extinction; N, estimated number of individuals present after 50 years.

Scenarios	Population P(E)	Population N	Western sub-Pop P(E)	Western sub-Pop N	Eastern sub-Pop P(E)	Eastern sub-Pop N
Two sub-populations, with revegetation: dispersal 1.98 animals each way per year, with fecundity increased by 20% and mortality reduced by 20%	0.00	209.9	0.00	167.6	0.00	42.3
Two sub-populations, with revegetation: dispersal 4 animals each way per year, with fecundity increased by 20% and mortality reduced by 20%	0.00	212.6	0.00	169.3	0.00	43.3
Two sub-populations, with revegetation: dispersal 10 animals each way per year, with fecundity increased by 20% and mortality reduced by 20%	0.00	212.9	0.00	169.5	0.00	43.4
Two sub-populations, with revegetation: dispersal 20 animals each way per year, with fecundity increased by 20% and mortality reduced by 20%	0.00	212.8	0.00	170.7	0.00	42.2

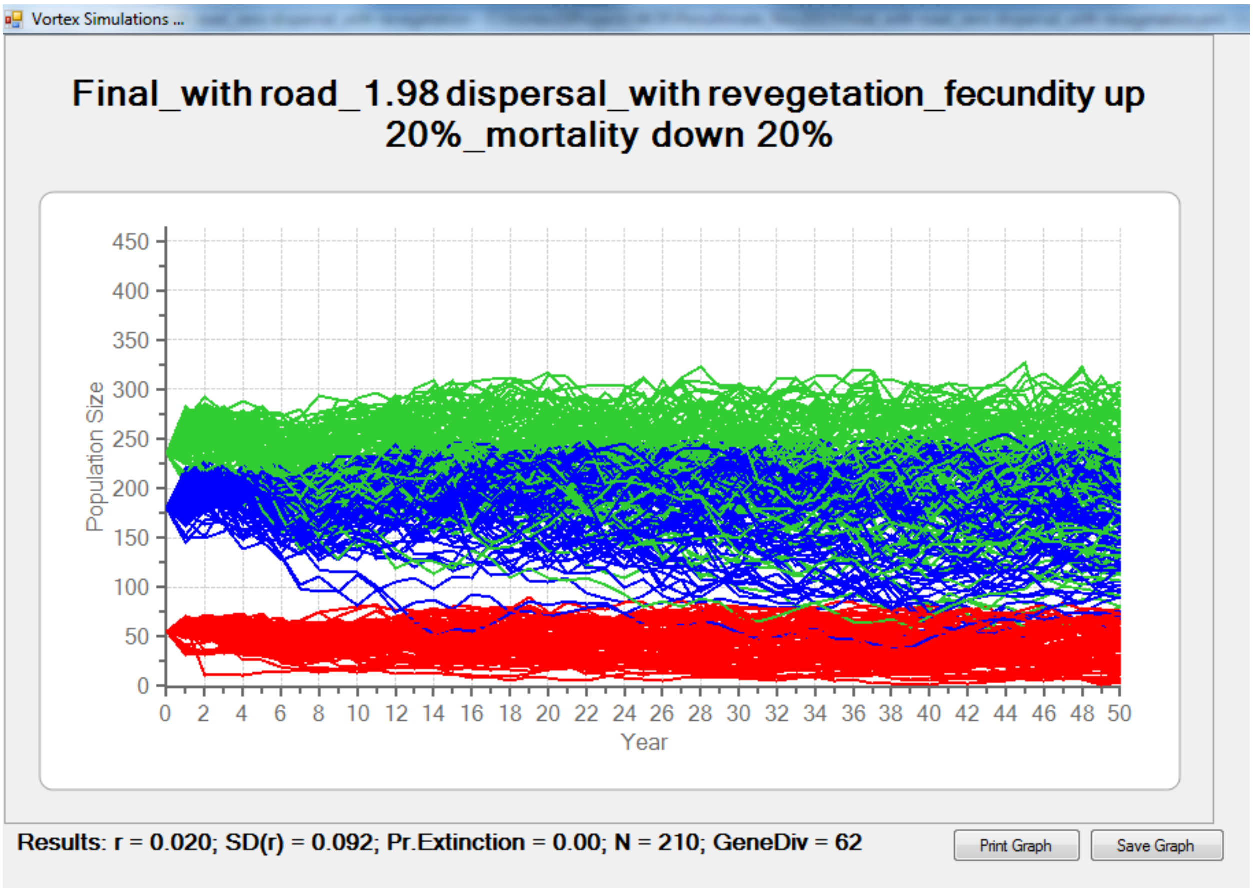


Figure 14: Stable Koala population projections over 50 years, with the proposed highway, when fecundity is increased by 20% each year and mortality is reduced by 20% each year across all age-gender classes. Other parameter estimates and model settings as for Figure 13, except that carrying capacity has been adjusted to reflect the steady increase in habitat availability that should result from new Koala food tree plantings in the study area. The smaller, eastern sub-population is indicated in red, the larger western sub-population in blue, and the total population in green.

7.8 Effects of the proposed road - using management to control mortality

Koala population viability in the study area was greatly enhanced by reducing mortality by eight young (<4 years) animals (5 males and 3 females) per year (Table 9; Figure 15). Under this scenario, the population was projected to decline very slowly, but still comprising approximately 170 animals after 50 years.

The easier target of reducing mortality by four young (<4 years) animals (2.5 males and 1.5 females) per year also had significant benefits to long-term Koala population viability with approximately 109 animals remaining after 50 years (Table 9; Figure 16).

These results suggest that management intervention to reduce Koala mortality due to vehicle strikes (e.g. fencing along the proposed road and other local roads within recognised hot spots), and local dog predation, have the potential to improve the prospects for this Koala population, compared to the current situation. It is unknown whether funding would be available without the road to undertake these important management actions (e.g. fencing).

Table 9: Impacts of the proposed road on the Ballina Koala population near Wardell when mortality is reduced by either 4 or 8 young animals per year.

All scenarios include the same demographic inputs, the same likelihood of two types of catastrophes, 2.85 animals emigrating out of, and 2.85 animals immigrating into, the study area, and changes to habitat carrying capacity to model the effects of losing habitat for five Koalas during road construction followed by the provision of new habitat for up to 41 Koalas. Data inputs from Neaves *et al*. (2015), Norman *et al*. (2015) and Phillips *et al*. (2015). Assumes the presence of inbreeding depression and lethal alleles.

P(E), probability of extinction; N, estimated number of individuals present after 50 years.

Scenarios	Population P(E)	Population N	Western sub-Pop P(E)	Western sub-Pop N	Eastern sub-Pop P(E)	Eastern sub-Pop N
Two sub-populations, with revegetation: dispersal 1.98 animals each way per year, with mortality reduced by 4 animals per year.	0.00	108.9	0.00	66.3	0.00	42.6
Two sub-populations, with revegetation: dispersal 1.98 animals each way per year, with mortality reduced by 8 animals per year.	0.00	170.7	0.00	112.0	0.00	58.6

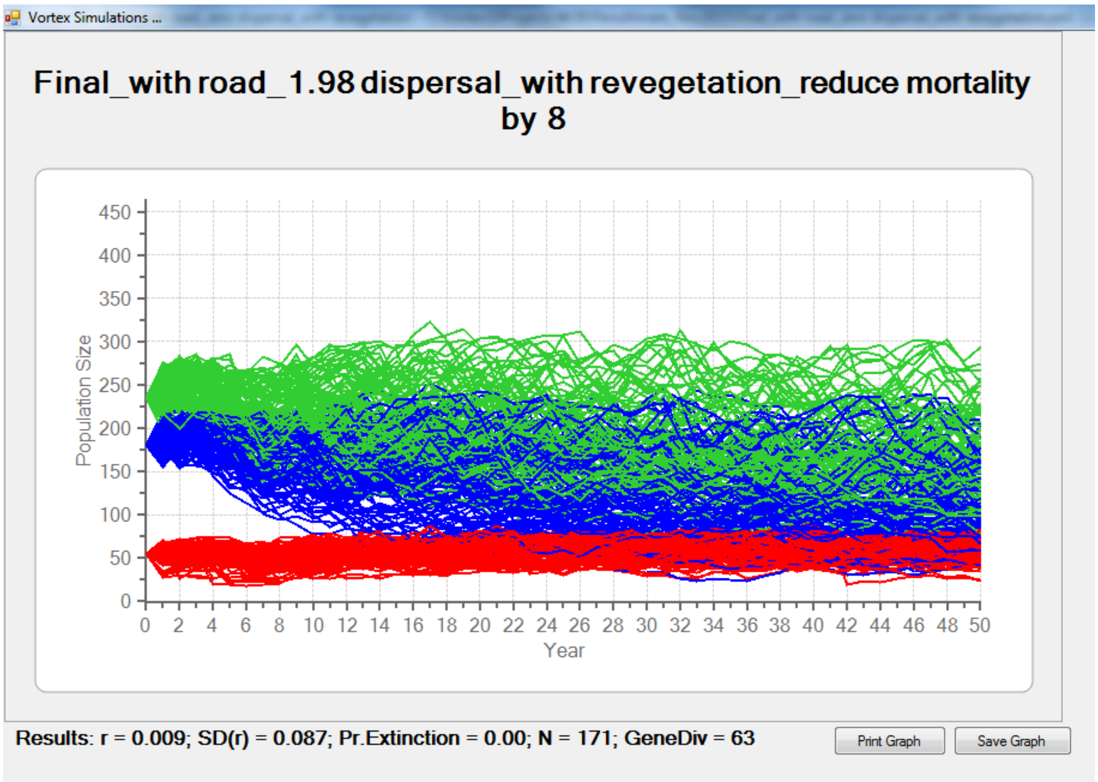


Figure 15: Koala population projections over 50 years, with the proposed highway, when mortality is reduced by 8 young animals per year through management intervention. Other parameter estimates and model settings as for Figure 14.

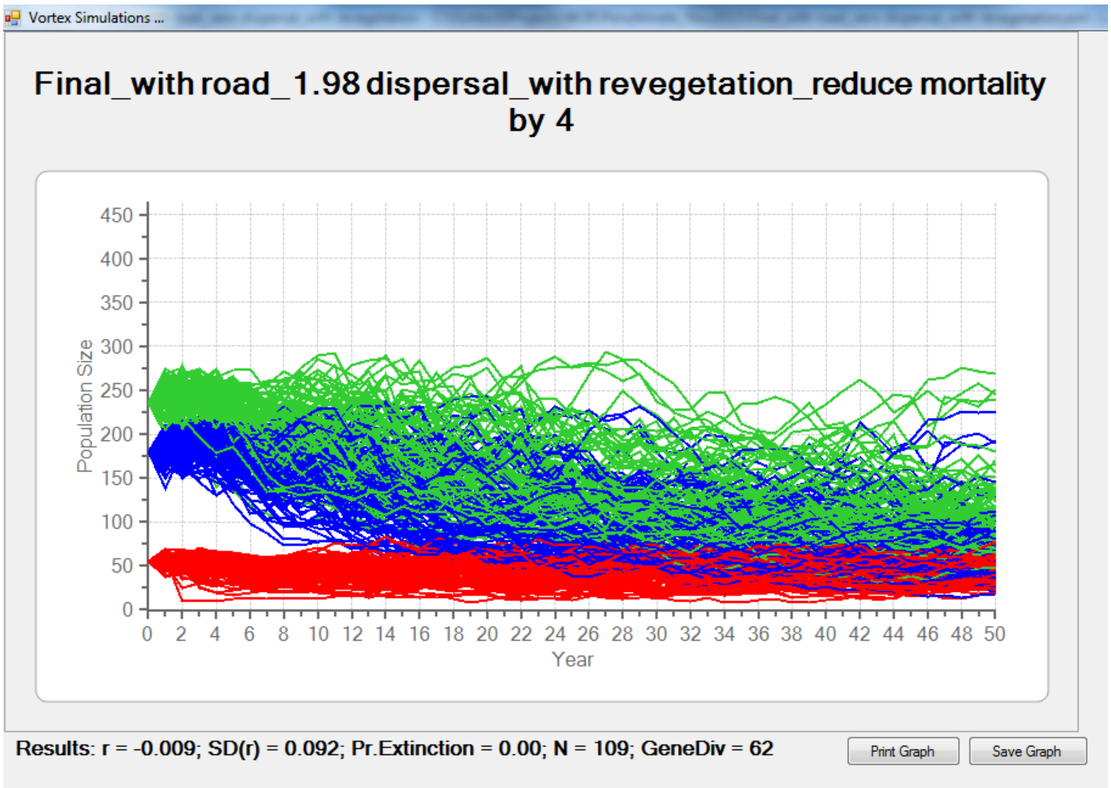


Figure 16: Koala population projections over 50 years, with the proposed highway, when mortality is reduced by 4 young animals per year through management intervention. Other parameter estimates and model settings as for Figure 14. The smaller, eastern sub-population is indicated in red, the larger western sub-population in blue, and the total population in green.

7.9 Effects of the proposed road – summary of impacts

The proposed road has the potential to cause a small adverse impact, reducing the projected population size over 50 years by 0-9.7%, depending on the rate of dispersal and assumptions about the effectiveness of the connectivity structures that will be provided (Table 10). Overall, dispersal rates did not have a major influence on the results (Tables 4-9).

The robustness of these findings was investigated using sensitivity tests of the impact of the proposed highway upgrade in relation to uncertainty in the estimates of demographic parameters (Table 10). These tests also included the impact of losing habitat for five Koalas following road construction. The sensitivity tests were performed by modelling an "optimistic" scenario (in which both mortality was reduced and fecundity was increased) and a "pessimistic" scenario (in which both mortality was increased and fecundity was reduced) for three different rates of dispersal.

These results (Table 10) showed that:

- Dispersal rate made little difference to the results (i.e. whether 100% is set at 1.98, or 10 or 20 animals dispersing each way per year).
- The impact of the road (i.e. the worst case scenario which modelled 40% of each of the above rates of dispersal) ranged between -0.5 to -9.4%, averaging -4.1%, across all scenarios.
- When the potential loss of habitat for 5 Koalas was taken into account, the impact ranged between -0.7—9.7%, averaging -4.8% across all scenarios.

Table 10: Sensitivity tests of the impact of the proposed highway upgrade in relation to uncertainty in the estimates of demographic parameters.

Scenarios represent variations on the standard parameter estimates for population fecundity and mortality (Phillips *et al*. 2015) and dispersal (Neaves *et al*. 2015, Norman *et al*. 2015), and they also incorporate the potential loss of habitat for five Koalas following road construction.

The road impact (%) is calculated from the modelled population projections resulting from each scenario, where "no road" represents 100% dispersal (i.e. 100% connectivity) and "with road" represents a "worst-case" reduction to 40% dispersal (i.e. 40% connectivity). Assumes the road is fully fenced to prevent additional mortality.

Scenarios / Impact assessments	Population projection (N) No road (100% dispersal)	Population projection (N) With road (40% dispersal "worst case")	Impact (%)
Dispersal = 1.98 animals each way/year			
No change in base rates (Table 4)	42.3	42.1	-0.5
- Including loss of habitat for 5 Koalas	42.3	42.0	-0.7
Optimistic: mortality reduced by 20% and fecundity increased by 20%	186.9	181.5	-2.9
- Including loss of habitat for 5 Koalas	186.9	180.1	-3.6
Pessimistic: mortality increased by 20% and fecundity decreased by 20%	19.5	19.2	-1.5
- Including loss of habitat for 5 Koalas	19.5	19.4	-0.3
Dispersal = 10 animals each way/year			
No change in base rates (Table 4)	43.4	41.7	-3.9
- Including loss of habitat for 5 Koalas	43.4	40.7	-6.2
Optimistic: mortality reduced by 20% and fecundity increased by 20%	187.3	186.3	-0.5
- Including loss of habitat for 5 Koalas	187.3	181.4	-3.1
Pessimistic: mortality increased by 20% and fecundity decreased by 20%	21.5	19.6	-8.7
- Including loss of habitat for 5 Koalas	21.5	19.4	-9.7
Dispersal = 20 animals each way/year			
No change in base rates (Table 4)	46.8	43.0	-8.1
- Including loss of habitat for 5 Koalas	46.8	42.3	-9.7
Optimistic: mortality reduced by 20% and fecundity increased by 20%	186.6	184.4	-1.1

- Including loss of habitat for 5 Koalas	186.6	185.0	-0.8
Pessimistic: mortality increased by 20% and fecundity decreased by 20%	22.3	20.2	-9.4
- Including loss of habitat for 5 Koalas	22.3	20.2	-9.2

7.10 Effects of the proposed road – summary of potential management responses

Management to reduce the incidence of vehicle strikes, dog predation, and potentially disease are required to improve the long-term viability of this population. The potential small adverse effects of the proposed road (section 7.9) could be reversed by the enormous potential to improve population projections through management intervention (Table 11). The form that these management interventions could take include:

- the provision of supplementary habitat after accounting for the loss of habitat for up to five Koalas resulting in a slight increase in projected population size (+0.5%)
- the provision of additional Koala-proof fencing along known road-kill hotspots in the study area, together with local dog control, so that Koala mortality is reduced by up to 4 or 8 animals per year resulting, with habitat supplementation, in large increases in projected population size (257%-404%), and
- improvements in the health of the population, potentially through the application of a new vaccine to counteract the effects of *Chlamydia* and so raise population fecundity resulting, with habitat supplementation, in a large increase in projected population size (267%) if breeding success could be raised by 20%.

Other combinations of these approaches could result in significant increases to the projected size of the population if mortality can be reduced and fecundity can be increased simultaneously (Table 11).

Table 11: Summary of the impacts of the proposed highway upgrade in relation to potential management interventions.

Scenarios represent variations on the standard parameter estimates for population fecundity and mortality (Phillips *et al*. 2015). They also incorporate the potential loss of habitat for five Koalas following road construction, and the provision of supplementary habitat for up to 41 Koalas by year 15. Management interventions also include full fencing along the proposed new road, with the potential to fence other roads (road-kill hotspots) in the study area.

The road impact (%) is calculated from the modelled population projections resulting from each scenario, where "no road" represents 100% dispersal (i.e. 100% connectivity) and "with road" represents a "worst-case" reduction to 40% dispersal (i.e. 40% connectivity).

Scenarios / Impact assessments	Population projection (N) No road (100% dispersal)	Population projection (N) With road (40% dispersal "worst case") plus management intervention	Impact (%)
Dispersal = 1.98 animals each way/year			
Effect of habitat supplementation, including the loss of habitat for 5 Koalas (Table 4 and Table 5)	42.3	42.5	+0.5
As above, but reducing mortality by 20% (Table 4 and Table 6)	42.3	90.8	+215
As above, but increasing fecundity by 20% (Table 4 and Table 7)	42.3	112.8	+267
As above, but reducing mortality by 20% and increasing fecundity by 20% (Table 4 and Table 8)	42.3	209.9	+496
Dispersal = 1.98 animals each way/year			
Effect of habitat supplementation, including the loss of habitat for 5 Koalas, with reducing mortality by either 4 or 8 young animals per year (Table 4 and Table 9)	42.3 42.3	108.9 (4) 170.7 (8)	+257 +404

7.11 Changes in genetic diversity

This section presents a summary of the number of alleles remaining within the Koala population under the range of modelling scenarios reported in sections 7.3-7.7. The scenarios reported in each section were conducted under a range of dispersal rates, and this accounts for the range in results. Projected population size was strongly correlated with the levels of genetic diversity in the population. Higher rates of dispersal in each scenario resulted in higher estimates of the number of alleles remaining in the population.

The effect of doubling the rate of dispersal from 1.98 to 4 individuals each way per year, to incorporate the effects of breeding rate being only 44.83%, resulted in a small increase in the number of final alleles in the population (4.89 to 4.90) even though the projected population size remained the same (i.e. 42.5 animals) (Table 5).

Table 12: Summary of the changes in genetic diversity resulting from the scenarios reported in sections 7.3-7.7.

Scenarios	Number of alleles remaining
1. Impact of the road under a range of dispersal scenarios, but not including the initial loss of habitat for five Koalas due to clearing for road construction and the provision of 130 ha of new Koala habitat.	4.81-4.96
2. Impact of the road under a range of dispersal scenarios, including the initial loss of habitat for five Koalas due to clearing for road construction as well as the provision of 130 ha of new Koala habitat	4.86-4.94
As for 2. above, but with mortality reduced overall by 20%	5.14-5.18
As for 2. above, but with fecundity increased by 20%	5.15-5.18
As for 2. above, but with both mortality reduced overall by 20% and fecundity increased by 20%.	5.34-5.35

Discussion

The projected population decline observed in all modelled scenarios is due to births not being adequate to offset deaths, regardless of any effects of inbreeding, the presence of the highway upgrade, or any of a range of mitigation efforts (i.e. Koala-proof fencing, connectivity structures, provision of new habitat) that might be implemented by RMS. This view was confirmed in email communications (18^{th} and 30th September 2015) between Dr Rod Kavanagh and Dr Bob Lacy (the author of the Vortex software). Dr Lacy stated "If these birth and death rates are correct, and if they continue to pertain to the local population, then it could only be sustained if there is a continual inflow of Koalas from other, healthier populations".

The primary issue is with the estimates of population demography, not with the presence of the road or the proposed mitigation efforts. It is unknown whether the demographic parameters, collected from a once-only snapshot sample, are truly representative of the population. Under the scenarios modelled, there are simply not enough Koalas to effectively utilise the new habitat that would be provided if the highway upgrade was constructed. The demographic estimates used in the models may well be correct but, if so, management attention needs to be focused strongly on measures that will either increase population fecundity, and/or reduce population mortality. As the models incorporating reductions in mortality by 4 or 8 young animals per year have shown, any efforts to reduce Koala mortality in the region will improve Koala population viability. This response is likely to occur primarily by increasing the numbers of breeding females in the population. Indeed, management interventions that result in an increase in fecundity by 20% are likely to be more effective in reversing the downward trend in this population than reducing mortality by the same amount, but if these measures can be combined, the population was projected to increase five-fold over current trends.

Dispersal was difficult to model initially for several reasons, although these problems were subsequently overcome. Firstly, dispersal is largely dependent on population density, although we have no clear understanding of these relationships. This issue was overcome using a range of plausible estimates derived from the genetics reports (Neaves *et al*. 2015, Norman *et al*. 2015) and from other field studies (Dique *et al.* 2003, Kavanagh *et al.* 2007). Secondly, initial attempts to model dispersal were expressed as percentage of the population, rather than as the number of animals dispersing. The application of dispersal as an equal percentage of each sub-population was problematic because the western sub-population was more than three times larger than the eastern sub-population. This effectively propped up the smaller sub-population (which was operating as a population sink by receiving three times as many individuals) at the expense of the larger sub-population. However, sensible comparisons were achieved when dispersal was treated symmetrically between the two sub-populations and expressed as the numbers of individuals dispersing per year. Thirdly, the impact of the road effectively rested upon comparisons between the rate of dispersal through the connectivity structures that are proposed, yet little published information is available about of these relationships. Assumptions about the effectiveness of the 26 connectivity structures (approximately one every 500 m along the proposed road) ranged from enabling 100% dispersal to a 'worst case' of only 40% dispersal. However, overall, dispersal rates did not have a major influence on the results.

Inbreeding was found to occur at very low levels in the population (Neaves *et al*. 2015, Norman *et al*. 2015), yet we took the conservative approach in our PVA modelling of assuming that inbreeding depression could present (O'Grady *et al*. 2006). This had the effect of reducing population projections by approximately 20-40% (unpublished preliminary results). The levels of genetic diversity in the population were strongly correlated with projected population size and the rate of dispersal. Therefore, any management interventions that serve to increase these two parameters are likely to result in improved genetic diversity within the population.

The proposed Pacific Highway Upgrade, by itself, is unlikely to contribute adversely to the viability of the Koala population near Wardell; the population is already in steady decline due to other factors (low breeding success, high mortality) and connectivity between the two sub-populations is not a big driver of population size. In the context of this steadily declining population, the proposed supply of 130 ha of new habitat for the Koala was unable to be fully exploited, but this could be reversed through management interventions to increase population size. Significant opportunities and benefits exist to reduce Koala mortality and thereby to assist an increase in fecundity as part of this Project. This could occur through the provision of a range of mitigation actions, including Koala-proof fencing along the proposed highway upgrade and at known Koala road-kill hotspots on other roads in the area. Similarly, efforts to limit the presumed incidence of disease in this population, if this had the effect of raising fecundity, would be highly beneficial to overall viability of the population.

Conclusions

The Conditions of Consent for this Project require that “the impacts to the Ballina Koala population are demonstrated to be acceptable within the Ballina Koala Plan”. No definition of “acceptable impact” has been provided, but in this study we have interpreted this to mean “no impact”. If by “no impact” we assume “no worse” than the status quo (i.e. no new road), then this study has shown that the proposed highway upgrade near Wardell (Section 10) could cause a reduction of between 0-9.7% in projected population size over the next 50 years. However, this small impact on the Ballina Koala population could be compensated for by the provision of Koala-proof fencing in the study area and by the establishment of 130 ha of supplementary new habitat. Indeed, the management responsibilities, actions and resources associated with this infrastructure have the potential to arrest the current steep decline in this population.

This study has shown that the Ballina Koala population is in desperate need of assistance because of its high mortality and low breeding success which will inevitably lead to its extinction if not addressed. Modest and achievable reductions in Koala mortality will improve the current unbalanced population structure and ensure that more females are available to increase population fecundity. Further work to investigate and reduce the incidence of disease in this population may be warranted if this has the effect of increasing fecundity.

All of the proposed connectivity structures and mitigation activities will benefit this population of Koalas, although some of them may not be fully utilised until the population decline is reversed.

Management Implications

Recovery of the Ballina Koala population is a responsibility for the whole community. The RMS has clear obligations to ensure that no Koala road-kills occur as a result of this Project, and indeed this organisation has committed to a fully-closed highway fencing system along the corridor, additional and enhanced connectivity structures, and the establishment of a minimum of 130 ha of new habitat for the Koala. In addition, the RMS is willing to undertake further work, such as fencing, at two known Koala hot-spots that occur on other roads in association with this Project (i.e. part of Wardell Road in the vicinity of the new highway, and part of the existing Pacific Highway north of Wardell to Coolgardie). The value of these additional measures, even if mortality could be reduced by just four animals per year, was clearly shown in the PVA modelling.

However, there are other road-kill hot spots, and other threats to this Koala population, that need to be addressed. The Bruxner Highway (a State road) and Bagotville Road (a Council road), each have significant Koala road-kill hotspots that require attention by the relevant authorities. Predation by domestic dogs is also likely to be a significant threat to Koalas in the study area and should be controlled. The local community and other government agencies have an important role to play in devising and implementing appropriate strategies to control dog predation on Koalas. This work could be done in conjunction with the management of a number of the offset properties adjacent to Section 10 that have been purchased by RMS on which predator control programs will be implemented. Koala mortality needs to be reduced by at least four to eight young animals per year to slow the rate of decline in this population.

The role of disease in potentially limiting population fecundity needs to be explored and understood. We know that disease is present in this population, but we do not know its true incidence and whether it is adversely affecting breeding success. Recent trials have shown that a newly-developed vaccine has the potential to protect wild Koalas from *Chlamydia* infections and to improve reproductive success in females (Waugh *et al*. 2015). Research funding is required to make the necessary assessments, and to consider whether medical intervention is required or indeed appropriate. Local community support may be the best way to obtain the research funds required.

Finally, regular and systematic long-term monitoring of the Koala population in the study area is required to determine whether recovery efforts and mitigation activities have been successful, and also to assess the accuracy of the PVA projections. RMS is responsible for initiating and funding such a monitoring program after road construction, as per the conditions of approval, but broader community involvement is required to maintain this program and to extend it in both space and time.

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Appendix 1 – Ecosure/Biolink Report

ecosure

biolink

Koala Population Survey

Woolgoolga to Ballina Pacific Highway Upgrade: Section 10 (Wardell to Coolgardie)

Final Report (Revised Edition)
December 2015

ROADS AND MARITIME SERVICES

ecology / vegetation / wildlife / aquatic ecology / GIS

Executive summary

This report presents findings from assessments of the distribution, density and demographics of a koala population inhabiting a notional study area of approximately 8,250 hectares in the southeast of the Ballina Local Government Area on the far north coast of New South Wales. Approximately 2,152 hectares of preferred koala habitat occurs within the study area, ranging from high carrying capacity habitats of forest red gum on coastal floodplain to lower carrying capacity grassy blackbutt forests on the more elevated ranges. The work described herein arose from a need for specific information concerning the study area's koala population that would provide necessary baseline input parameters for Population Viability Analysis modelling to help evaluate the potential impacts on koalas of the conditionally-approved Woolgoolga to Ballina Pacific Highway Section 10 upgrade.

To assist the PVA process a demographic profile of the population was constructed from a subset of 51 koalas sampled from across the study area. The overall picture that emerged from this process was of a small and widely dispersed population that appeared to be primarily sourced from high carrying capacity red gum forests on the floodplains and swamp mahogany forests on coastal sands in the south of the study area. Tooth-wear/age class frequency data indicated a population that was at demographic equilibrium. A predominance of older animals in the north implied that birth rates in the north and recruitment levels from the west and south were not sufficient to offset mortalities. Six of the 51 koalas that were screened for purposes of demographic profiling were obtained as a direct consequence of vehicle-strike.

Despite the trend toward demographic equilibrium at the population level, a skewed sex-ratio evidenced by lower numbers of males implied a measure of male-biased mortality that was most notable amongst the sub-adult and older male tooth-wear classes. Reproductive output amongst adult female koalas was estimated to be approximately 43% annually across the population. Consistent with the trend towards older animals in the north, reproductive output by female koalas was higher in the south of the study area. Clinical expression of disease amongst male and female koalas was relatively low.

The annual mortality rate of koalas across all tooth-wear/age-frequency classes was estimated from the capture and screening program at approximately 10% of total population size. To better understand factors contributing to koala mortality, the detail of 472 koala-calls received by the Lismore-based Friends of the Koala over a 26-year period from 1989 to 2014 were examined. Two hundred and seventeen of the 472 records related to a koala mortality, 75% of which were from within the study area. Excluding instances where the reason for a given koala mortality was either not recorded or unknown, natural causes accounted for approximately 10% of known mortalities annually. In contrast, domestic dog attacks and vehicle-strikes accounted for more than 50% of all koala deaths annually, the latter involving significantly higher numbers of males than females and thus providing one explanation for the male biased mortality estimated by the capture and screening program. Mortalities (including euthanasia) due to disease were also high, the data over time notable for the presence of two peaks 15 – 17 years apart, the basis for which appears to be operating independently of other mortality drivers and factors such as climate.

Koala density across the study area was reassessed using direct count techniques at forty-six 250 metres x 40 metres (one hectare) transects, the majority of which were sites in which koalas were also counted in 2013 using a different sampling protocol. Eleven koalas were recorded during the transect searches, three of which were recorded within survey transects. The 2013 survey estimated koala density to be 0.12 koalas ha^{-1}, whereas the 2015 transect surveys returned a density estimate of 0. 07 koalas ha^{-1}. These data were pooled to create a refined density estimate of ~ 0.09 koalas ha^{-1}, thus enabling a more refined population estimate for the Important Population Focal Area of approximately 196 koalas to be derived.

Field surveys to better understand the distribution and extent of koala activity, as well as identifying areas of unoccupied habitat to inform potential translocation sites, involved assessment of 76 field sites at approximately 350 metre intervals, all of which were located in forested areas east of the conditionally-approved Section 10 upgrade alignment and west of the existing Pacific Highway. Subject to some qualifications, the resulting model indicated that the distribution of significant koala activity (and thus resident koala populations) was typically focused in areas of higher quality habitat. Koala density in this smaller study area was estimated at approximately 0.096 koalas ha^{-1} with an occupancy rate estimated to approximate 40% of available habitat. Areas of high carrying capacity but otherwise unoccupied habitat occurred in several areas. Potential impacts of road construction on the local koala population were accentuated by the locations of significant koala activity, the modelling serving to inform a conclusion that the home range areas of 10 – 14 koalas will be affected to varying degrees based on estimated koala densities within the forest areas that would be directly impacted by the road alignment; occupied habitat areas currently linking the eastern and western population cells in the area between Thurgate's Lane and Buckombil Mountain Road will also be impacted.

Based on an estimated population size of 196 koalas, an annual mortality rate of approximately 10% and that death due to vehicle-strike and attacks by domestic dogs collectively account for more than half of all koala mortality annually, a long-term, secure outlook for the study area's population appears uncertain. Given that the numbers of koalas killed on an annual basis by vehicle-strike is a function of population size (i.e. greater numbers of individual movements and more home range areas near roads when population numbers are higher), striving to offset the potential impacts of road construction by increasing population size alone will be largely ineffective if factors contributing to current mortality rates cannot be reduced substantively.

To enable forward projections relating to the potential impacts of the conditionally approved upgrade and associated issues of koala management to be objectively evaluated, population-specific baseline input parameters and other considerations relating to the PVA process were derived from the component studies comprising this report. The purpose of this information is to enable the current conservation status of the population to be objectively ascertained, as well as informing various management scenarios that may need to be developed and appraised if impacts are to be deemed 'acceptable' over the 50 year time frame specified by the Federal Government's consent condition.

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Acknowledgements

Recognition and acknowledgement of individuals and/or organisations who helped us to carry out this project can be viewed at http://www.biolink.com.au/projects/koala-pva-project.

We also acknowledge field support from Maria Matthes, George Kordas, Bob Johnson, Emily Hatfield, Jim Dick, Cam Weetman, Karlee Taylor and Caitlin Weatherstone, as well as Chris Allen and Rob McKinnon from the Office of Environment and Heritage. We are especially grateful to Maria Matthes who not only assisted with locating, capturing and processing koalas (day and night), but also tirelessly collected and followed-up on koala sightings from the local community, and provided a devoted and inspirational role model for all who worked on the project. At the Ecosure end we also thank Marianne Donoghue, Beth Kramer, Noela Hamilton, Katherine Richardson, Phil Shaw, Jeff McKee and Matt Smyth.

We extend our sincere thanks to the Friends of the Koala (FoK) volunteers and leadership team and in particular Lorraine Vass, Pat Barnidge and Susannah Keogh for their 24-hour support and advice whenever (and at whatever time of the night) we encountered koalas with signs of disease. The FoK have a vital ongoing role in the management and recovery of koalas throughout the region.

We would like to acknowledge and thank the Nyangabul people and the Jali Local Aboriginal Land Council for their permission and support in accessing survey sites on Jali lands, and sincerely hope that burabi’s will survive on these lands for future generations to enjoy and treasure.

The final report has also benefited from review comments from Associate Professor Jonathan Rhodes (UQ) and Dr. Rod Kavanagh (Niche Consulting), as well as feedback from Roads and Maritime personnel Bob Higgins, Scott Lawrence, Julie Ravallion and Simon Wilson.


PART 1
Introduction

1.1 Introduction

In May 2012, the koala was listed as a threatened species throughout Queensland (QLD), New South Wales (NSW) and the Australian Capital Territory (ACT) under the Commonwealth Government's *Environment Protection and Biodiversity Conservation* (EPBC) *Act 1999*. In NSW, koalas are listed as a Vulnerable species under the *Threatened Species Conservation* (TSC) *Act 1995*, with the viability of free-ranging koala populations decreasing in most areas due to a variety of threatening processes including ongoing habitat loss, modification and fragmentation, stress-related disease, bushfire, vehicle-strike and domestic dog attacks. While there is a substantive body of scientific literature informing this knowledge (Threatened Species Scientific Sub-committee 2012), there is yet no evidence demonstrating successful population recovery.

Since the mid-1990s the NSW Government has been committed to a progressive upgrade of the Pacific Highway to a safer dual carriageway standard between Newcastle and the QLD/NSW border. As a component of this commitment, NSW Roads and Maritime Services (Roads and Maritime) have been investigating a range of highway upgrade options in the area between Woolgoolga, to the north of Coffs Harbour and Ballina, south of Byron Bay. The proposed alignment (referred to as Section 10) of the existing Pacific Highway between the Richmond River and Ballina deviates from the existing highway via a new bridge over the Richmond River approximately six kilometres south of Wardell and traverses inland before re-joining the existing alignment three kilometres north of Wardell.

A koala habitat and population assessment across the entire Ballina Local Government Area (LGA) was completed in 2012/13 (Biolink 2013)[1], constituting the first comprehensive assessment of koalas and their habitat undertaken for the LGA. Amongst other things the BKS identified an area in the southeast of the LGA that was considered to support a nationally important population of koalas. This assertion was corroborated by historical records dating back over a century, together with indigenous knowledge and extensive areas of continuous occupancy by koalas over at least 40 – 50 years/six consecutive koala generations. The disparity between the habitat occupancy rate predicted by the historical analysis and the results of the field survey component implied a decrease in the extent of habitat supporting resident koala populations over recent years. This was supported by an overall low koala density estimate of 0.09 to 0.13 koalas ha^{-1}, the latter applying to areas wherein koala activity was known to occur. The envisaged Section 10 upgrade passes through the area occupied by the aforementioned population, the traverse directly impacting upon the eastern most population cell identified by the BKS, thus having the potential to displace a number of koalas.

The Woolgoolga to Ballina (W2B) Pacific Highway upgrade (including Section 10) was approved by the NSW Government in June 2014. In August 2014, the Federal Environment Minister similarly approved the W2B upgrade, albeit with a number of additional conditions including a need for further work to be undertaken on koala populations inhabiting the area to be traversed by Section 10. Specifically, the conditions required use of Population Viability Analyses (PVA) to be undertaken over a 50-year time frame in order to better understand long-term impacts (of road construction) on the area's resident koala populations. PVA was also to be utilised to examine the likely impacts of different management scenarios that might

[1] Hereafter referred to as the Ballina Koala Study (BKS)

be employed to reduce adverse consequences to a measure that was deemed ‘acceptable’.

The purpose of this report is to detail results from an examination of factors of relevance to koala population viability within the Important Population Focal Area[2].

Along with a separate genetic profiling study, the analyses and resulting outcomes from this examination are intended to inform specific components of the PVA process required by the Federal Environment Minister including:

a) the demographic structure of the koala population inhabiting the IPFA, including details of sex-ratios, reproductive output, age-class frequencies and associated mortality rates,

b) analyses of koala mortality data for the IPFA, including the extent of incidental harvests due to domestic dog attack and vehicle-strike,

c) a re-assessment of koala abundance (density) within the IPFA with a view to further refining estimates of population size previously obtained by the BKS, and

d) obtaining further knowledge of koala distribution east of the Section 10 alignment for the purpose of revising the extent and type of potential ameliorative measures, as well as informing possible population management programs such as translocation should the proposed upgrade proceed.

1.1.1 The study area

The Ballina LGA covers approximately 49,200 ha on the far north coast of NSW between the Byron Shire to the north, Lismore City to the west and the Richmond Valley LGA to the south. Within this area, a notional boundary considered to support an ‘important population’ (i.e. the IPFA) for purposes of the EPBC Act was identified by the BKS. The ‘notional’ IPFA encapsulates an area of 8,247 ha in the southeast of Ballina LGA known locally as the Blackwall Range between Wardell and Alstonville, as well as associated lowland areas that include the localities of Meerschaum Vale, Pimlico, Wardell and Bagotville. Figure 1.1 illustrates the location of the IPFA in the context of the Ballina LGA.

[2] Herein referred to as IPFA

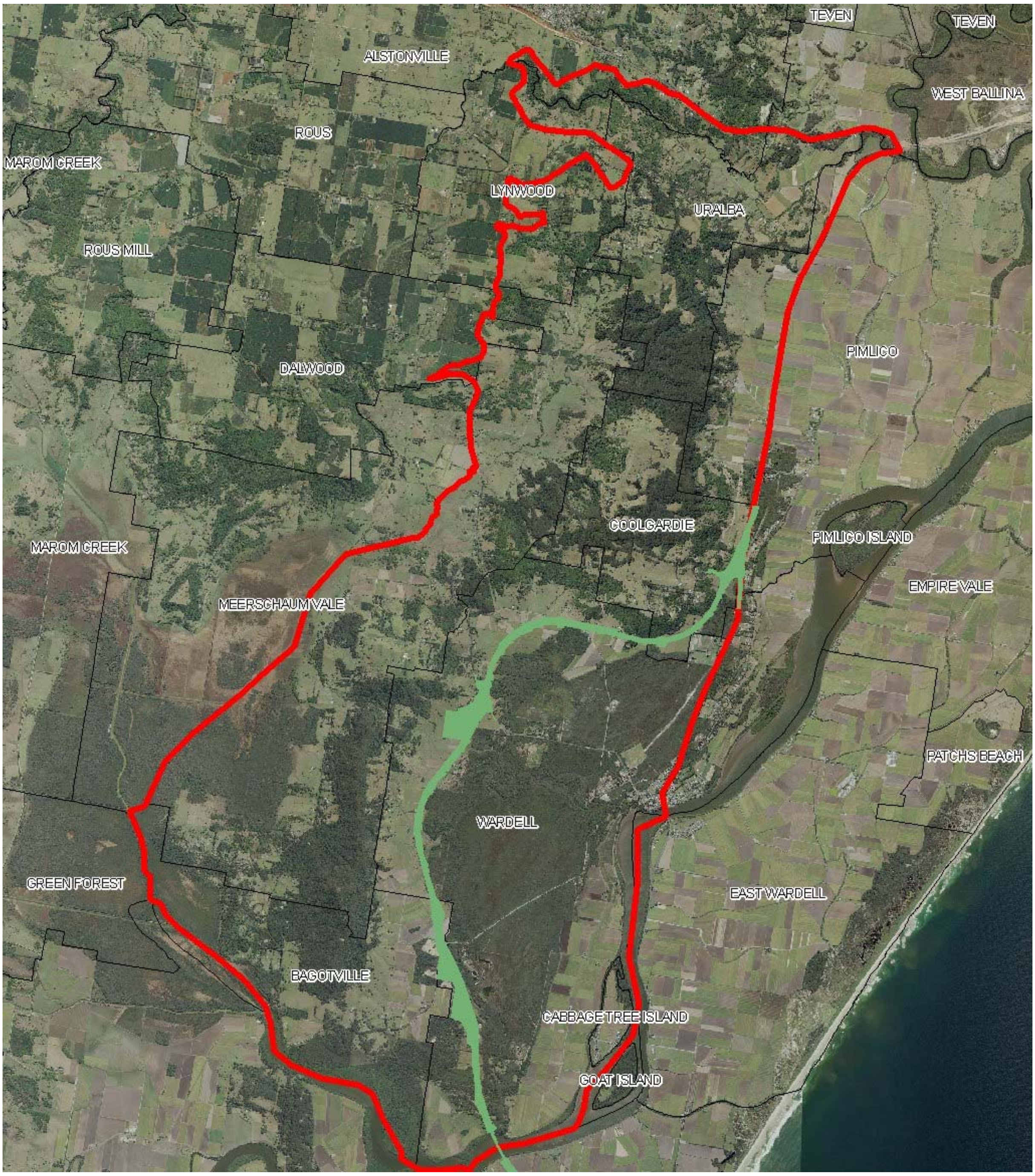

Figure 1.1 Location of the notional IPFA (red polygon) in the southeast of the Ballina LGA, including the envisaged alignment (green, linear polygon) of the conditionally approved Stage 10 W2B Pacific Highway upgrade

1.1.2 Extent of habitat and koala carrying capacity

Based on results of the BKS, suitable koala habitat within the IPFA is primarily determined on the basis of the distribution and abundance of the following preferred food tree species:

- tallowwood (*Eucalyptus microcorys*)
- swamp mahogany (*E. robusta*)
- forest red gum[3] (*E. tereticornis*).

Based on knowledge regarding the distribution and abundance of these three species, coupled with information regarding the influence of underlying soil landscapes on the palatability of preferred food tree species for koalas, approximately 2,152 ha of preferred koala habitat (PKH) has been mapped within the IPFA based on BKS koala habitat mapping (Table 1.1).

Table 1.1 . Estimated extent and carrying capacity of Preferred Koala Habitat classes within the IPFA. Carrying capacity estimates are based on mean values determined by other studies in comparable habitat types (Source: Phillips and Forsman 2005[4]; Phillips, Hopkins and Callaghan 2007[5]; Phillips and Allen 2012[6]; Phillips and Allen 2014[7])[8].

Koala habitat class	Area in IPFA (ha)	Carrying capacity
Primary Koala Habitat	96	0.63 koalas ha^{-1}
Secondary (Class A)	578	0.42 koalas ha^{-1}
Secondary (Class B)	808	0.23 koalas ha^{-1}
Secondary (Class C)	670	< 0.1 koalas ha^{-1}

The preceding data can be used to estimate a carrying capacity for the IPFA of ~ 556 koalas. However, a population of this size within the IPFA would be unsustainable and typified by evidence of over-browsing with nowhere for dispersing animals to colonise. Results from the aforementioned reports wherein populations were assessed as large (i.e. >350 koalas) and at demographic equilibrium imply that approximately 50% of available habitat (52.34% ± 5.15% - 95% CI) will be occupied at any one point (Phillips and Forsman 2005; Phillips, Hopkins and Callaghan 2007; Phillips and Allen 2014); this figure is now utilised as a sustainable management/recovery benchmark in most recently prepared Koala Plans of Management in NSW. Thus we consider the maximum number of koalas that could sustainably occur within the IPFA landscape for PVA purposes to be ~ 291 koalas (i.e. 556 x 0.5234).

[3] Includes the naturally occurring *E. robusta* x *E. tereticornis* hybrid.

[4] An ecological overview of koalas and their habitat on the Innes Peninsula, Port Macquarie NSW.

[5] Koala habitat and population assessment for Gold Coast City LGA.

[6] Koala conservation in the south-east forests: assessment of the need for and feasibility of a population augmentation program.

[7] Strzelecki Ranges Koala Survey.

[8] Copies of these reports can be provided if required.

1.1.3 Stochastic considerations

(i) Drought

Archival Drought Statements prepared by the Bureau of Meteorology's National Climate Centre (http://www.bom.gov.au/climate/drought/) indicate that the far north coast of NSW (including the IPFA) experienced several periods of serious rainfall deficiency between 1990 and 2014. Rainfall is considered to be deficient if it is within the lowest 10% of historical totals (well below average) for any given period of three months or more. Protracted serious to severe rainfall deficiencies were recorded for the IPFA between December 2001 and January 2004, and again from December 2013 to November 2014. These data indicate that serious local rainfall deficit conditions have occurred over at least three of the last 14 years thus enabling an estimate of the annual probability of occurrence of a drought episode to be ~ 21%.

Drought conditions impact upon tree health and habitat quality, koala morbidity and reproductive output within the IPFA. Impacts on koalas and their habitat during local drought conditions are likely to be greatest in elevated ridge areas, particularly those on less structured, shallower soil profiles with relatively low moisture-holding capacity where trees have less access to ground moisture reserves. Based on our observations from a number of local government areas, we hypothesise that the impact of such conditions within the IPFA may equate to failure in successful reproductive output in ~ 10% of breeding females over ~ 32% of available habitat (i.e. 700[9] ha of ridgeline habitat out of 2,152 ha) during serious rainfall deficit years. The impacts on reproduction and survival are expected to be small with proportional estimates of 0.1 and zero respectively. Our observations within the IPFA and in other areas suggest that these effects are restricted to ridge tops, populations during drought conditions faring better in low-lying areas on alluvial deposits and quaternary sand sheets. This could arguably be one of several contributing factors already affecting the current distribution and conservation status of the koala population across the IPFA.

(ii) Fire

Fire history is not well documented within the IPFA, oral history recalling a single, wildfire event in the Ngunya Jargoon IPA approximately 35 - 40 years ago (Marcus Ferguson pers comm), while NSW NPWS records describe a localised wildfire near Uralba in the northern parts of the Blackwall Range during the 1970s.

While the preceding information implies a relatively low frequency/probability of occurrence of ~ 3%, the impacts of such an event will vary depending on exactly where the wildfire occurs. Within the Ngunya Jargoon IPA for example, it is likely that the greater proportion of the habitat would be impacted with a correspondingly high impact on koala survival and reproduction. In contrast, a wildfire event in the Uralba – Coolgardie area would likely remain quite localised with a lesser impact. In consideration of such extremes we speculate that on average such a wildfire would likely affect no more than 10% of the IPFA in a single event, with associated impacts on reproduction and survival for PVA purposes estimated as severe to moderate (proportional estimates of 0.85 and 0.6 respectively).

[9] Estimated on basis of 100 m wide area of influence along ~ 35 km of ridgeline in elevated areas of IPFA.

1.1.4 Burabi and the Nyangabul people

The study area is located within the boundaries of the Nyangabul people of the Bundjalung Nation. The Nyangabul people have a long association with and connection to the koala, which they know as burabi and have traditional customs and stories associated with this connection that go back generations. Jali Local Aboriginal Land Council own and manage the greater proportion of land within the area to be impacted, while the associated Ngunya Jargoon Indigenous Protected Area supports the bulk of suitable koala habitat to the east of the conditionally approved Section 10 alignment.

PART 2
Population demographics

2.1 Objective

Detailed information on population structure, reproductive rates and other parameters influencing population dynamics and viability are fundamentally important components of the PVA process.

The primary objective of this part of the study was to obtain data on the current demographic structure of the IPFA koala population, including the distribution and proportional representation of age-classes, associated mortality rates, sex-ratio and numbers of reproducing females.

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2.2 Methods

2.2.1 Koala capture, processing & data collection

A 2.5 km x 2.5 km grid-base overlay was used to guide the capture and screening program to ensure to the maximum extent possible that a uniformly distributed survey and capture effort was achieved by ideally restricting the number of captures to no more than five – seven koalas per grid cell. Subject to landowner approvals, koalas were captured by both pole and flag or by trap (Phillips 2011)[10], anaesthetised and clinically assessed. During each assessment, the koala's sex, weight and body condition score were determined, clinical signs of disease recorded and samples collected for later analysis (see below). While the presence of pouch or dependent back-young was recorded, juvenile koalas were not included in the screening program.

2.2.2 Age-classing

Tooth-wear classes (TWCs) of Gordon (1991)[11] were used to allocate individual koalas to a population cohort. To assist PVA considerations we adapted the upper 95% confidence interval from Table 2 of Gordon (1991) to define the maximum age in years for each TWC; when rounded to the nearest whole number this approach enables partitioning into annual age increments as outlined in Table 2.1.

Table 2.1 Partitioning of koala tooth-wear classes (TWC) and corresponding stages (TWS) into annual age increments. Max. age = maximum age (in years) as indicated by the upper 95% confidence interval in Table 2 of Gordon (1991).

TWC	TWS	Max. age	Age in years
1	P4A	-	1
2	P4B	2	2
3	P4C	3	3
4	P4D	5	4 - 5
5	P4E	7	6 - 7
6	P4F, M1f-h	10	8 - 10
7	P4F, M1F, M2g-i	-	10+

[10] Development of a lightweight, portable trap for capturing free-ranging Koalas *Phascolarctos cinereus*. *Australian Zoologist* **35(3)**, 747 – 749.

[11] Estimation of the age of the koala *Phascolarctos cinereus* (Goldfuss) (Marsupialia Phascolarctidae) from tooth-wear and growth. *Australian Mammology* **14**, 5 -12.

2.2.3 Female reproductive status

The reproductive status of females was ascertained using a four-tiered assessment as follows:

(i) No pouch young present nor evidence of recent occupancy or lactation,

(ii) Pouch-young present,

(iii) Back-young present, or

(iv) Neither pouch-young nor back-young present, but evidence of recent/ongoing lactation (functioning mammary gland and elongated teat).

2.2.4 Koala health & welfare

In addition to physical examination and visual assessments of disease status and health, ocular and urogenital swabs were also taken to assist ongoing koala *Chlamydia* research programs, fur samples for a toxicology study, and ear tissue samples for Koala Retro-virus (KoRV) analysis and genetic profiling. Where appropriate, each koala was micro-chipped and/or identified with individually numbered Wireless Identification Device (WID)[12] or FoK ear-tags to assist sample referencing and longer-term monitoring. Once fully recovered from the anaesthetic, each koala was released back into the tree from which they had been captured or an adjacent smaller tree.

In instances where a koala was either determined upon capture to be moribund, assessed as being in poor health or otherwise expressing chronic disease symptoms, it was transported to the FoK Care Centre at Lismore. Access to some koalas was also facilitated through the FoK hotline/network; koalas killed by domestic dogs or motor vehicles in the study area were considered to be part of an incidental harvest resulting in demographic data and tissue samples that could be included in the data collection/screening program.

2.2.5 Central tendency measures

The majority of data associated with the capture and screening program were enumerative. Hence data were assumed to follow a binomial/poisson distribution and unless otherwise specified, the Standard Deviation was estimated using the following term:

$\sigma = \sqrt{}pq/n$ (Eqn 1)

Where:

σ = standard deviation of the sample

p = the sample proportion

q = 1 – p

n = total sample size

12 Optionally fitted to koalas captured within 500m of a site initially considered for a land bridge at a location between Old Bagotville Road and Richmond River.

2.2.6 Mortality rates

Mortality rates for male and female cohorts were estimated from age frequency data using the basal formula of Heincke (1913) modified for koalas by dividing the initial mortality rate estimate for each Tooth Wear Class (TWC) by the maximum age (Table 2.1 refers) as follows:

$M_i = (\Sigma N_{(i\ldots)} - N_i / \Sigma N_{(i\ldots)}) / A_{max}$[13] (Eqn 2)

Where:

M_i = mortality rate for TWC "i"

ΣN = total number of koalas in TWC (i…) for each gender cohort

N_i = number of koalas in cohort "i"

A_{max} = maximum age represented by the upper 95% confidence interval estimate for the corresponding TWC in Table 2 of Gordon (1991).

Estimates of the Standard Deviation associated with environmental variation (SD_{EV}) for each cohort-based mortality rate (*M*) were derived by multiplying *M* by the Coefficient of Variation (CV) associated with the mean numbers of deceased koalas documented by the FoK over the 26 year period 1989 – 2014 (Part 3 refers).

[**Note:** there are several assumptions inherent in the calculation of mortality estimates that rely upon age-frequency data, not all of which (e.g. a stationary population with stable age structure) might hold for a given population, thus limiting the reliability of the associated estimates. Other techniques are also available, all of which share similar assumptions or require other types of data not collected by this study. Despite its relative straight-forward and intuitive nature, the approach adopted herein is one of the better approximations we have examined in terms of mirroring actual mortality rates in free-ranging koala populations.]

[13] The use of the maximum age is required to accurately enable annual mortality estimates to be calculated given that each of the TWC groupings in Gordon's 1991 approach encompass more than one year.

2.3 Results

Subject to property access restrictions, the majority of forested habitat within the IPFA was searched for koalas. There were several instances (e.g. Coolgardie through to Uralba) where koalas were sighted, but either land-owners did not approve of their capture or the animals were not catchable due to forest structure and topography.

Data from 51 koalas including one skull were obtained over the period from early December 2014 through to mid - June 2015. Of these, three koalas were the victim of domestic dog/fox attack, six the result of vehicle-strike, two captured by hand when observed crossing open ground and the remainder captured using pole and flag or trapping techniques. The distribution of captures ranged across the IPFA from Lynwood and the Bruxner Highway in the north to Pimlico, Wardell and Bagotville in the south (Figure 2.1). Despite extensive searches only two koalas, both males, were located and captured within 500 m of the initially considered location for a land bridge at a site between Old Bagotville Road and the Richmond River. Three of the captured koalas had been previously ear-tagged by FoK. Only one of the 51 specimens could not be aged, this being a female koala killed by vehicle-strike and whose skull was crushed as a consequence. A summary of the capture data is provided in Appendix 1.

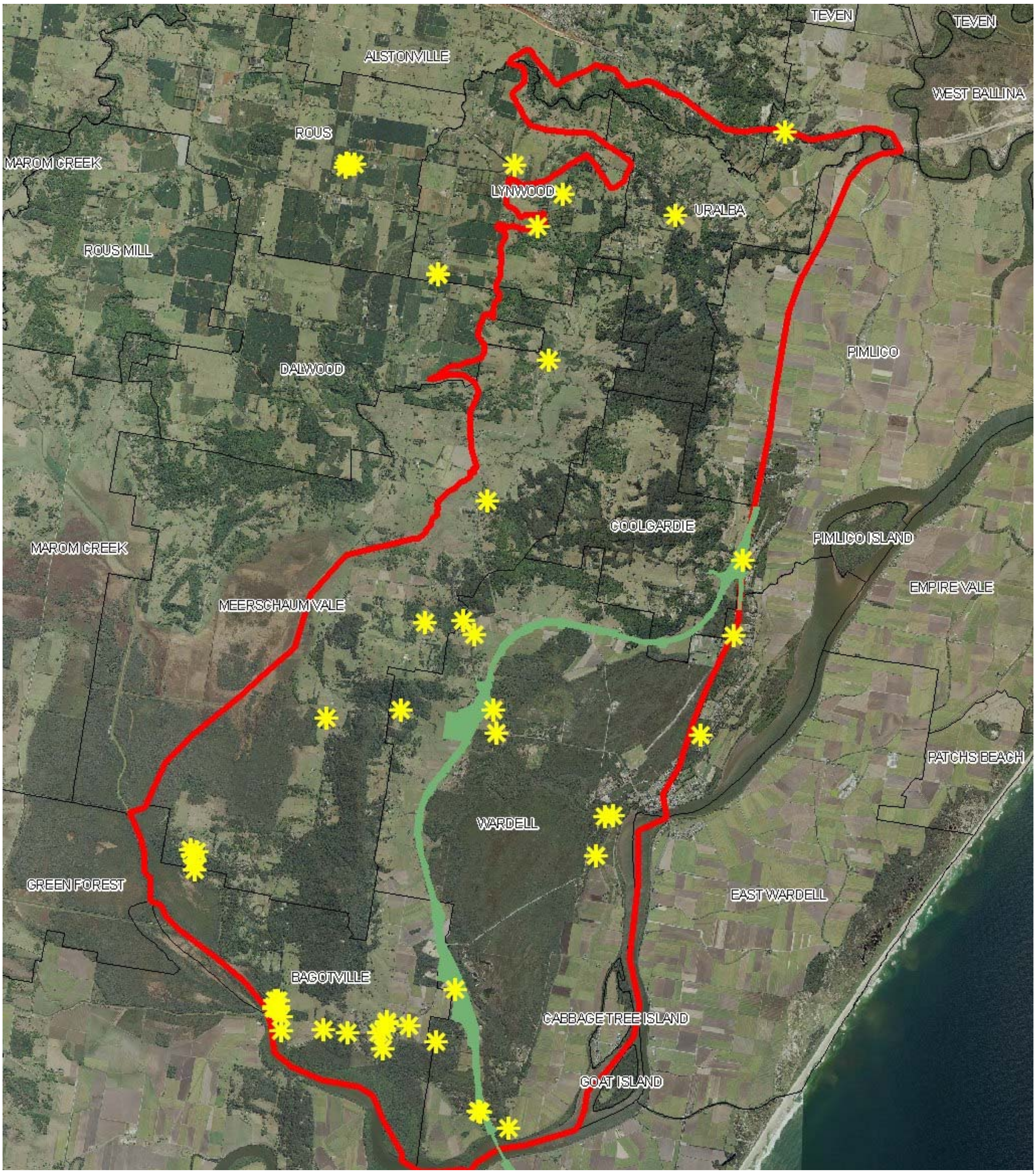


Figure 2.1 Localities of 51 koalas (yellow highlighted asterisks) informing the demographic profile for the IPFA. Further details for each sample are provided in Appendix 1.

2.3.1 Population structure and composition

Six TWCs (2-7) were represented amongst the captured koalas, the frequencies of which are illustrated in Figure 2.2. We estimated the ecological window represented by the samples to cover a minimum period of 10 years, this being the difference between the oldest animal in the sample (10+) and the youngest (small, unfurred pouch young no more than several weeks of age) observed during the screening program. The aggregated data-set implies a population trending towards a predominance of older age-classes. This pattern was most notable in the north of the IPFA where older age-class animals formed the more commonly-captured cohort.

Figure 2.2 Tooth-wear/age-class frequency distribution of IPFA koalas.

The gender ratio at population level was strongly female-biased with female koalas being almost twice as common as males (Ratio: 1.68 females for every male). Figures 2.3 and 2.4 illustrate the age-frequency histograms for male and female koalas, both of which imply approximately normal distributions.

2.3.2 Reproductive output

Evidence of reproduction was recorded in 13 of the 29 adult females represented in TWCs 3 – 7, this outcome enabling an estimate of 44.83% ± 9.27% (SD) of adult female koalas breeding annually. Reproductive activity in females was restricted to TWCs 3 – 5. Joey ages ranged from pouch young that were a few weeks of age up to advanced back young. As a subset for female koalas generally, breeding female koalas were more commonly recorded in the south of the IPFA (Figure 2.5).

Figure 2.3 Tooth-wear//age-class frequency distribution of female koalas within the IPFA.

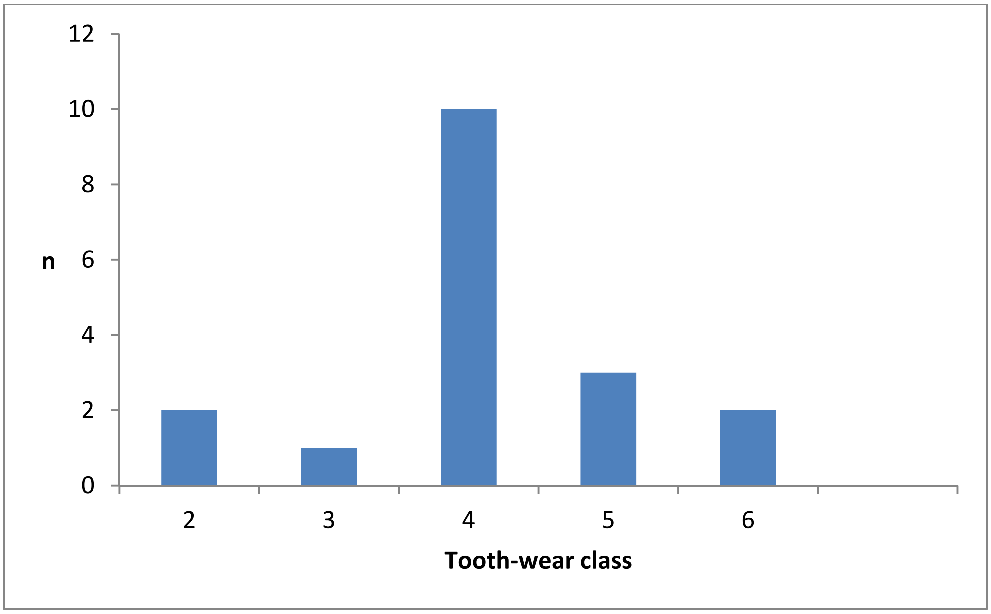


Figure 2.4 Tooth-wear/age- class frequency distribution of male koalas within the IPFA.

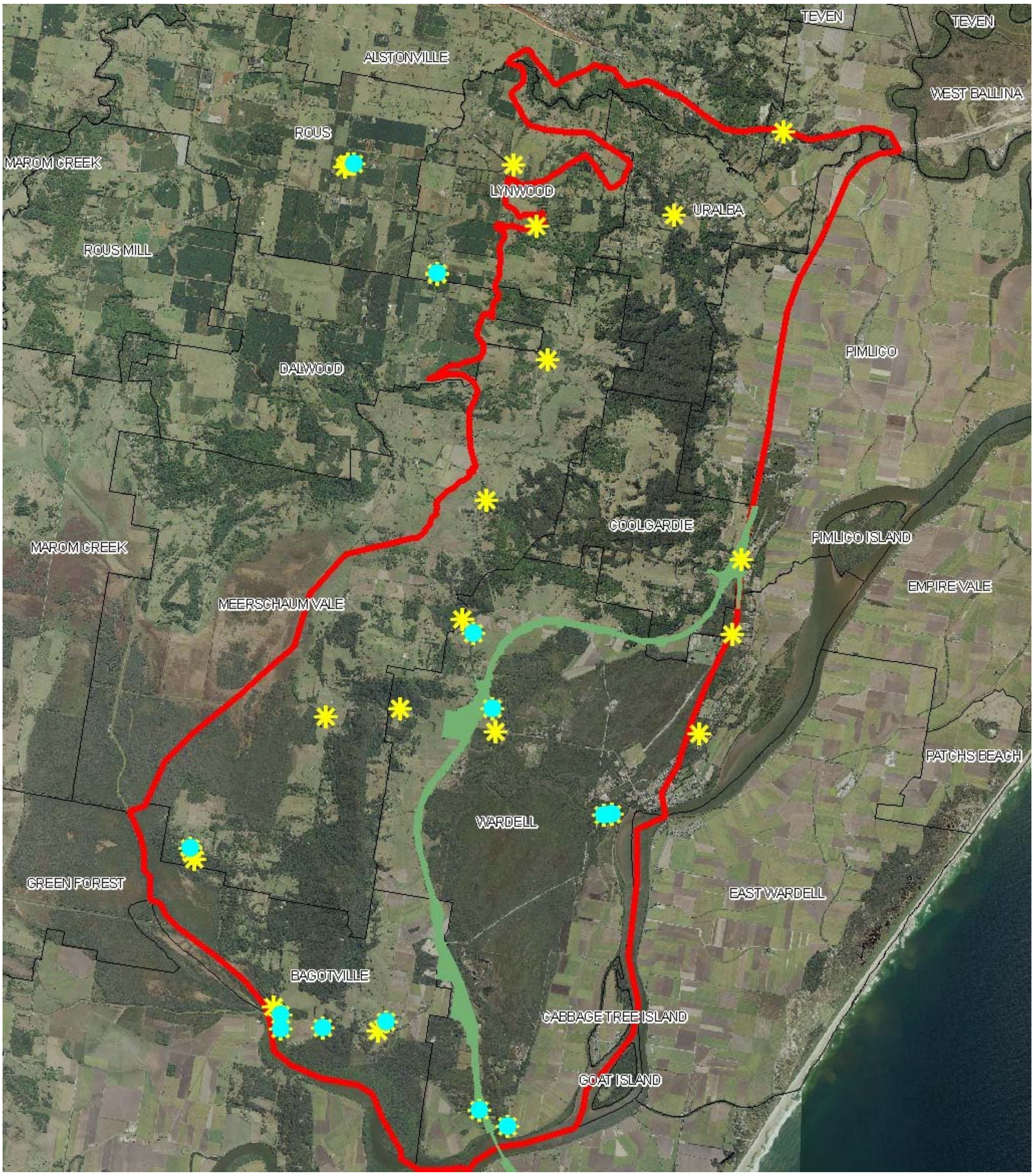


Figure 2.5 Locations of 13 breeding female koalas (blue highlighted asterisks) as a subset of all adult female koalas (n = 30) captured and screened within the IPFA.

2.3.3 Mortality rates

When averaged across all cohorts, annual mortality within the IPFA population was estimated at 9.94% ± 8.91% (SD). Table 2.2 provides a breakdown of the IPFA mortality estimates by gender-based cohort.

Table 2.2 Estimated mortality rates (M) for each of the six TWC cohorts represented in the IPFA koala population. Estimates are based on a sample of 32 female and 18 male koalas. Information in columns TWC and TWS reflect corresponding tooth-wear classes and associated scores of Gordon (1991) respectively. SD_{EV} is an estimate of the standard deviation of the mortality estimate due to environmental variation.

Age (years)	TWC	TWS	M(%)	SD_{EV}
Females				
1	1	P4A	19.7	11.63
2	2	P4B	19.7	11.63
3	3	P4C	26.21	15.48
4	4	P4D	7.34	4.33
5		P4D	7.34	4.33
6	5	P4E	5.37	3.17
7		P4E	5.37	3.17
8	6	P4F	2.76	1.63
9		P4F	2.76	1.63
10		P4F	2.76	1.63
Males				
1	1	P4A	19.45	11.49
2	2	P4B	19.45	11.49
3	3	P4C	30.56	18.05
4	4	P4D	4.3	2.54
5		P4D	4.3	2.54
6	5	P4E	6.29	3.71
7		P4E	6.29	3.71
8	6	P4F	2.92	1.73
9		P4F	2.92	1.73
10		P4F	2.92	1.73

2.3.4 General observations

Notwithstanding a low koala encounter rate over the greater proportion of the IPFA, in general terms captured koalas were in good condition, while chronic clinical expression of disease appeared to be primarily restricted to localities between Meerschaum Vale and Lynwood in the northern half of the IPFA. Of nine koalas taken to the FoK Care Centre during the course of the capture and screening program, four were euthanized on humanitarian grounds because of the extent of wasting/trauma/disease. Of the remaining five, two died upon arrival and were subsequently assessed as the consequence of an earlier vehicle-strike and sepsis respectively, while the remaining three (one adult and two sub-adults) were admitted for treatment at the Care Centre.

Evidence from the literature indicates that inbreeding depression and associated loss of genetic diversity will reduce resilience and evolutionary capacity, lower fitness and so increase the risks of extinction. Few morphological indications of potential inbreeding such as micro-cephaly, reduced body size/weights and/or other anomalies (e.g. undescended testicles) were observed amongst captured koalas. On this basis it is not considered likely that inbreeding depression will be a significant factor impacting on current baseline input parameters. Nonetheless, this parameter should be reappraised once the genetic analysis has been completed and may also need to be considered in conjunction with scenario modelling to accommodate potential future isolation effects associated with the conditionally-approved Section 10 upgrade.

Data from nine of the 51 koalas were obtained as a direct consequence of incidental mortalities due to domestic dog/fox attack (*n* = 3) and vehicle strike (*n* = 6) respectively. While it is acknowledged that the numbers of koalas killed by these two processes are grossly under-reported, an estimation of the actual numbers involved would assist the PVA modelling process. While some indication of the extent to which vehicle-strikes are under-reported is provided in Part 3, it is noteworthy that in terms of attacks by domestic dogs, local landholders in the Lynwood area have also advised of at least five unreported mortalities of sub-adult and adult koalas (including breeding females) over the five-year period 2010 – 2014, none of which are recorded in the publicly available databases.

2.3.5 Key outcomes

Demographic data was obtained from a subset of 51 koalas from the population inhabiting the IPFA, with koalas killed by vehicle-strike comprising almost 12% of those animals sampled over the course of the study. The population profile that emerges is of a relatively small and widely dispersed population primarily sourced from high density/carrying capacity red gum forests on the floodplains and swamp mahogany forests on coastal sands in the south-southwest of the study area.

The capture data indicates a population that is characterised by an approximately normal age-class distribution. Despite this, older animals were more commonly encountered in the north of the IPFA, implying that recruitment levels from the west and the south may not be sufficient to offset mortalities.

The female bias in the population is atypical compared to other populations we have assessed. The reasons for this are unclear, however given the results outlined in Part 3, we consider that this may be a reflection of male-biased mortality amongst sub-adult and older male cohorts. Clinical expression of disease was relatively low, constrained primarily to a small number of koalas in the north of the IPFA, while reproductive output by female koalas was higher in the south.

The average annual mortality rate across all cohorts was estimated at 9.94% ± 8.91% (SD) of the total population size.

PART 3
Analysis of FoK mortality data

3.1 Objectives

Understanding the factors that contribute to koala mortalities and so influence distribution, abundance and population trends over time is important if long-term, sustainable management outcomes are to be realised. The preceding demographic profile revealed a koala population with an average annual mortality rate of approximately 10% of total population size, the results implying amongst other things a high measure of male-biased mortality. The objective of this Part is to further investigate mortality data for the IPFA through analysis of records maintained by the FoK.

3.2 Methods

3.2.1 Data collection and partitioning

Records related to ‘koala-calls’ received by the Lismore-based FoK 24-hour rescue hotline for the Ballina LGA were provided for analyses. Amongst other things, attributes associated with each of these records include the date, location and underlying reason for the call, as well as the outcome, typically reported as either advice/record only, in-care, released, relocated or dead (including euthanasia).

At the LGA level, records were examined in order to describe general trends over time, followed by a partitioning and analyses of koala mortality data and the localities/contexts with which such data was associated. Because mortality data was of specific interest to the intended outcomes of this report, records that related to dead koalas were partitioned into four primary categories as detailed below. In instances where uncertainty relating to the cause of death was reflected in the mortality being attributed to more than one category, the score was partitioned accordingly (i.e. a cause of death may have been listed as ‘dog attack/road-kill’ in which case both categories of incidental mortality received a score of 0.5 each).

Further details for each of the four primary mortality categories are as follows:

A **natural** mortality was attributed to those records of koalas that were identified as having died from causes such as being aged and/or wasted (including instances of age and/or wasting associated with expression of disease). Juvenile koalas found dead on the ground or at the base of trees were also considered to have died from natural causes, as were those predated upon by a native animal or presumed to have died from misadventure such as falling from a tree.

A **symptomatic** mortality was assigned to deaths described as due to wet-bottom, conjunctivitis and/or blindness, organ failure and cancer/lympho-sarcoma, all of which tend to be typically associated with Chlamydiosis or Koala Retro-virus (KoRV) and generally considered attributable to stressors from anthropogenic related factors such as historical habitat loss or modification and/or genetic factors such as inbreeding.

An **incidental** mortality was one caused by vehicle-strike or domestic dog attack, or where injuries would suggest that either of these was the most probable cause of death (e.g. presence of a broken jaw, puncture marks).

A mortality due to **unknown** causes was attributed to records where the associated information in the database was generally insufficient to unambiguously identify a direct cause of death. Such records were subsequently discarded for the purposes of most of the analyses reported herein.

3.2.2 Data analysis

3.2.2.1 Koala-calls

Unless otherwise alluded to by the results herein, the majority of the derived data associated with callouts were enumerative. Because of this, most data were assumed to follow a poisson distribution. Hence, unless otherwise specified, the Standard Deviation was estimated using the formula previously detailed in Part 2.

Frequency histograms were used to show trends in the number of koala-calls over time, between months and by season, with regression analysis utilised to examine any potential relationships between time and the number of koala-calls that were received.

3.2.2.2 Mortality data

Mortality data was initially partitioned in terms of whether the record occurred within the IPFA or elsewhere in the LGA, a *t*-test was used to examine differences in the annual numbers of dead koalas between the two areas. Chi-square analyses were then used to examine differences in mortality frequencies for each of the primary categories between the two constituent parts of the LGA. Thereafter analyses were restricted to only those records that occurred within the IPFA. Frequency histograms were again used to show trends in the mortality data over time.

Amongst other things, a requirement of koala population management is knowledge regarding the extent to which mortalities beyond those that occur naturally in the population will influence population trends over time. Such knowledge is especially important where mortalities may be a direct result of human activity in the form of either vehicle-strike or domestic dog attack. An estimate for the number of koala deaths due to vehicle-strike that are not reported can be derived from data collected by Phillips and Fitzgerald (2014)[14] whereby 12 of the 27 records compiled for a 50 km length of the Pacific Highway between Chinderah and Ewingsdale in Byron and Tweed LGAs were not otherwise included in publicly available databases. Such knowledge enables an estimate of the number of unreported koala road-kills of 44.44% ± 18.74% (95% Confidence Interval). At least six koalas were killed by vehicle-strike in the IPFA during the course of the demographic study; while numbers of previously unreported domestic dog attacks were also obtained (Part 2 refers).

In addition to incidental harvests of koalas due to vehicle-strike and domestic dog attack, increased mortalities may also result from elevated disease levels in areas where individual animals and populations are subject to traumas or where populations remain isolated from each other through historical and ongoing processes of habitat fragmentation. In such cases, inbreeding arising from a long period (i.e. many koala generations) of isolation may also have some bearing on both the stress response and incidence of disease and associated mortality levels. However, depressed fecundity and/or an increase in mortalities due to disease may also be a part of a naturally occurring, density–dependent phenomenon that enables population regulation.

[14] A review of koala road-kill data and issues relating to underpass use by koalas: Pacific Highway upgrades from Clothier's Creek to Ewingsdale, NSW. Final report to NSW Roads and Maritime.

3.2.3 Standardising road-kill

Within the IPFA road-kill data were standardised across Council and State-managed roads in terms of the minimum number of koalas being killed per kilometre per year; this was done by initially defining the distance of the road traverse along which the road-kill records were distributed and then averaging the number of koalas killed per kilometre along these sections of road annually over the time period for which records were available. Any additional NSW Wildlife Atlas koala road-kill records along these roads for the same time span as that covered by the FoK records were also considered in these calculations.

In order to investigate for potential correlations between any peaks in disease-related mortality and climatic fluctuations, rainfall data was obtained from historical records available through the Australian Government's Bureau of Meteorology website. Rainfall over the period to which the records related was estimated by averaging annual rainfall figures from up to nine weather stations; Ballina Airport, Meerschaum Vale (Bardon), Meerschaum Vale (jbd), Coraki, Woodburn, Evans Head (bombing range), New Italy, Lismore Airport and MacLean's Ridges.

3.3 Results

3.3.1 Koala-calls

The FoK database contained details of 472 koala calls that were received over a 26-year period from 1989 to 2014, the frequency of calls over this time period (Figure 3.1) averaged approximately 18 annum^{-1} (Mean no of calls: 18.15 ± 9.72 (SD), range 4 - 38), increasing significantly over time ($r^2 = 0.411$, $F = 16.75$, $P < 0.001$). For each of the key mortality drivers of domestic dog attack, vehicle-strike and symptomatic disease, the annual proportion of koala-calls related to these categories did not vary significantly over the 26-year period to which the records relate (dog attack: $r^2 = 0.008$, $F = 0.1134$, $P = 0.7413$; vehicle-strike: $r^2 = 0.0106$, $F = 0.2029$, $P = 0.6575$; disease: $r^2 = 0.0034$, $F = 0.0752$, $P = 0.7865$).

Rainfall over the 26-year period to which the records relate averaged 1,588 mm annum^{-1} (Standard deviation: ± 414 mm; Range: 1035 – 2808 mm), the driest years occurring in 1992 and 2002, the wettest in 1999, 2008 and 2010 (Figure 3.2).

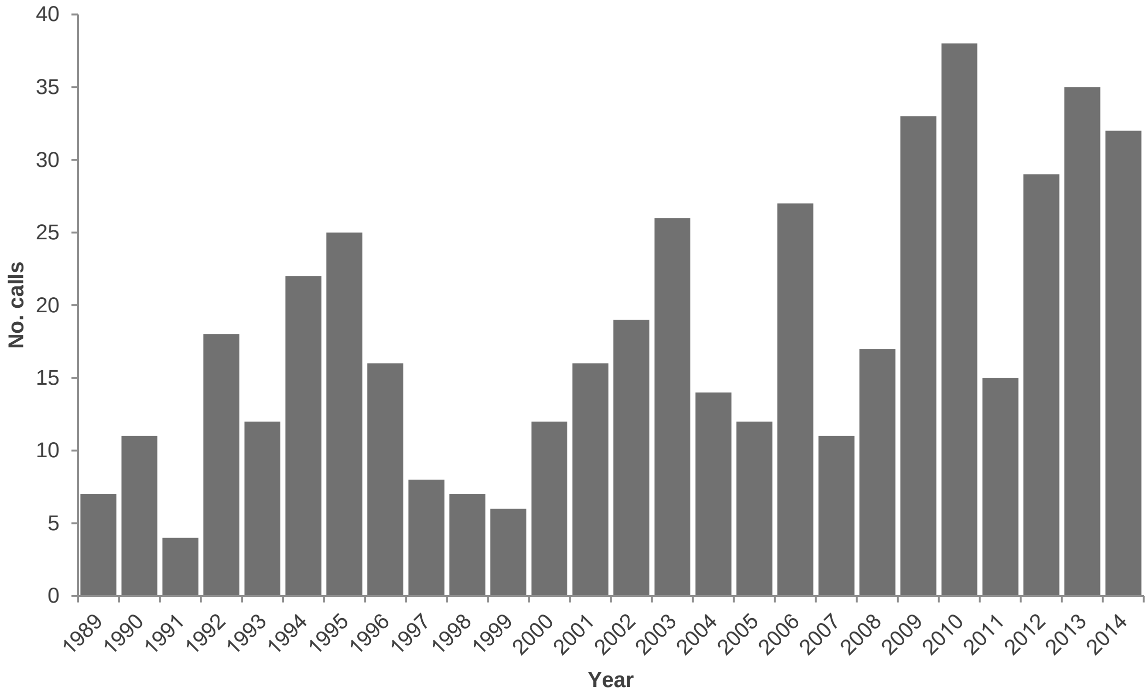


Figure 3.1 Numbers (n) of koala-calls received by FoK from across the Ballina LGA between 1989 and 2014.

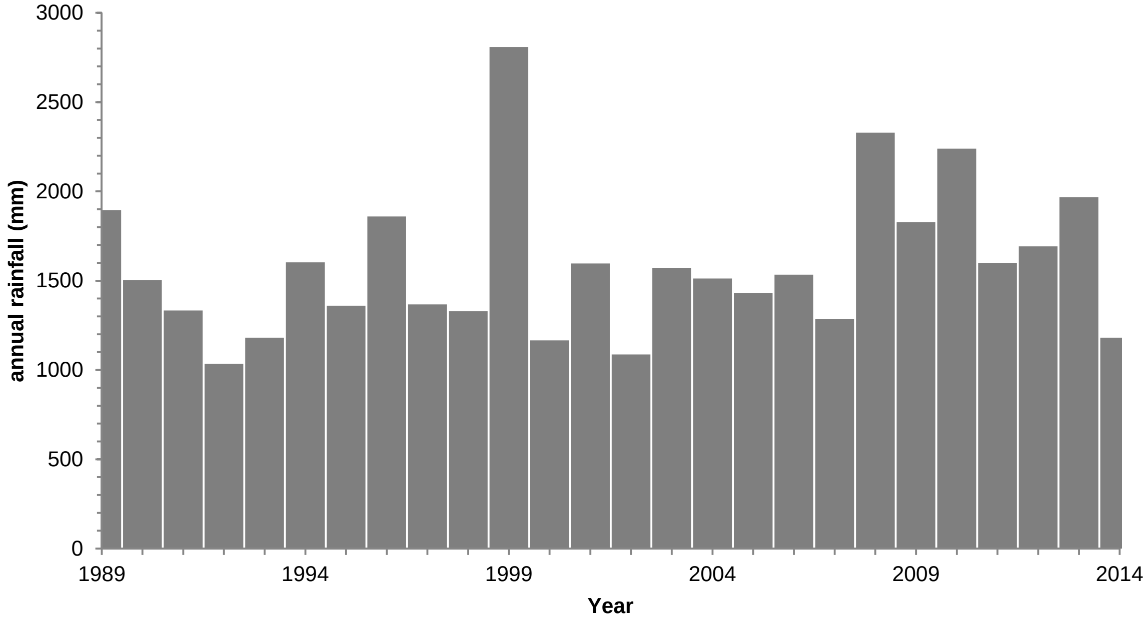


Figure 3.2 Annual averaged rainfall totals for the area covering the IPFA over the period 1989 – 2014 (Source Australian Bureau of Meteorology). Totals have been estimated from a series of up to nine weather stations in and immediately adjoining the study area.

Koala-calls were most frequently received between the months of July and January, peaking over Spring. All cohorts were represented in the call-out records, including orphaned joeys, females with pouch or back-young and aged koalas. Male koalas were more likely to be the subject of a koala call than were female koalas (X^2 = 23.273, 1_{df}, P < 0.001). Figure 3.3 illustrates trends in the frequency of koala calls by month and season.

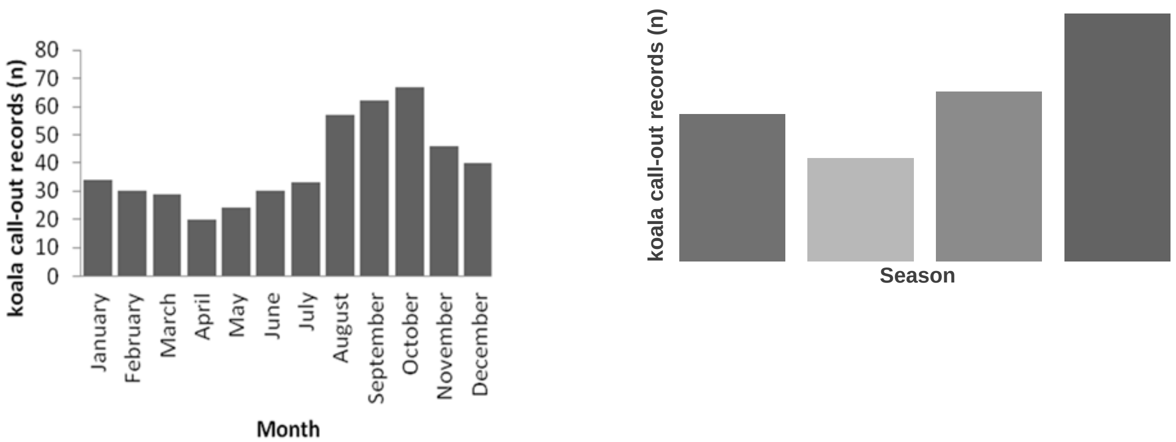


Figure 3.3 Monthly and derived seasonal trends in FoK koala-calls from across the Ballina LGA between 1989 and 2014.

3.3.2 Mortalities across the LGA

Of the 472 calls responded to by FoK, approximately 46% (*n* = 217) resulted in the death of the koala. Across the LGA, known mortalities averaged approximately 8 koalas a year [Mean = 8.34 ± 4.45 (SD), the numbers of which while trending upwards over time appear to have remained relatively stable (r^2 = 0.0406, *F* = 1.0145, *P* = 0.3239). Approximately 75% of all known mortalities (162/217) were from within the IPFA, the mean number of known annual mortalities therein (Mean = 6.23 ± 3.68 (SD) significantly higher than elsewhere in the LGA (6.23 *vs* 2.12 known mortalities annum^{-1}; Levene's Test: *F* = 3.364, 25_{df}, P < 0.01; *t* = 5.006, 39_{df}, P < 0.001).

Excluding 68 mortalities where the primary cause of death was recorded as 'unknown', the major contributors to koala mortality across the Ballina LGA were disease and vehicle-strike respectively, both of which collectively accounted for 69% of cause-known koala mortalities. Attacks on koalas by domestic dogs accounted for a further 20% of known mortalities, the remaining 11% recorded as being due to natural causes. Significantly, incidental mortalities due to vehicle-strike and domestic dog attack collectively comprised more than 50% of all cause-known koala mortalities annually. Table 3.1 further partitions the primary causes for koala mortalities in terms of the IPFA and the remainder of the LGA.

Table 3.1 . Numbers of known koala mortalities and attributed causes across the Ballina LGA from 1989 – 2014, partitioned in terms of occurrence either within the IPFA or elsewhere in the LGA. Numbers of mortalities attributable to unknown causes are provided in brackets but are not included in totals.

Area/Cause	Dog Attack	Road	Natural	Disease	Unknown	Totals
BLGA (part)	3	19.5	5	12.5	(15)	**40**
IPFA	22	35	12	40	(53)	**109**
BLGA (all)	**25**	**54.5**	**17**	**52.5**	(68)	**149**

With the exception of attacks on koalas by domestic dogs which were significantly higher in the IPFA when compared to the rest of the LGA, there were no significant differences between the IPFA and the remainder of the LGA in terms of the frequencies of mortalities attributable to other causes (Vehicle-strike: X^2 = 2.999, *P* = 0.083; Natural: X^2 = 0.064, *P* = 0.8 and Disease: X^2 = 0.578, *P* = 0.447).

3.3.3 IPFA mortalities

3.3.3.1 Natural causes

Database records for the IPFA contained 12 records where the cause of death was considered to be due to natural causes, this category comprising 11.01% ± 3.0% (SD) of all mortalities where cause of death was known.

3.3.3.2 Incidental causes

Domestic dog attacks

Database records for the IPFA contained 22 records of known koala mortalities where the cause of death was assessed as being due to an attack by a domestic dog, this category comprising approximately 20.18% + 3.85% (SD) of all incidental mortalities within the IPFA where the cause of death was known. Despite what otherwise appears to be a downward trend in the number of fatal domestic dog attacks on koalas being reported over the last decade (Figure 3.4), regression analysis did not attribute a measure of significance to the trend at this point in time (r^2 = 0.0076, F = 0.084, P = 0.7769).

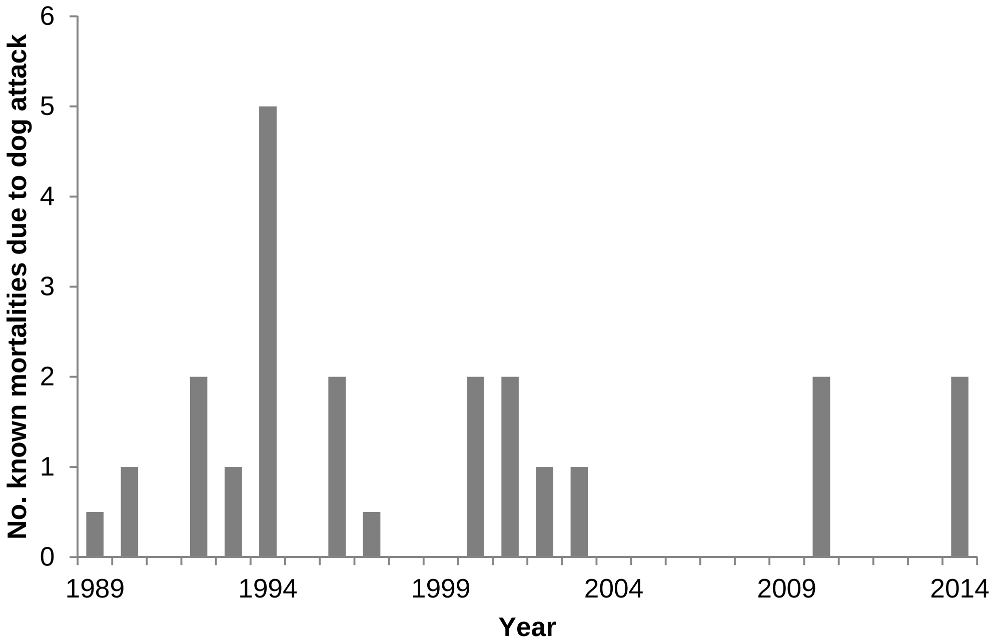


Figure 3.4 Numbers of known koala mortalities across the IPFA that were attributed to attacks by domestic dogs over the time period 1989 – 2014.

Attacks on koalas by domestic dogs were most commonly reported from the Meerschaum Vale area (36%) followed by Coolgardie (18%), Wardell (14%), Bagotville (14%) and thereafter other localities including Uralba, Lynwood and Cabbage Tree Island. Based on the raw data there did not appear to be a significant difference in the frequencies of male (n = 10) and female koalas (n = 12) that were known to have died as a consequence of domestic dog attack.

The minimum number of annual koala mortalities within the IPFA that can be estimated as directly attributable to attack by domestic dogs was 1.64 ± 0.33 (SD) koalas annum^{-1}.

Vehicle-strike

Following disease, vehicle-strike was the second largest contributor to koala mortality within the IPFA, responsible for 32.11% ± 4.47% (SD) (n = 35/109) of all cause-known koala deaths between 1989 and 2014. Death from vehicle-strike was most commonly recorded from the localities of Meerschaum Vale (34%), Wardell (26%) and Alstonville (26%). The roads in these areas where vehicle strike was most commonly recorded include the Bruxner Highway to the east of Alstonville down to the intersection with the Pacific Highway (23%), Bagotville and Wardell Roads (21% and 13% respectively) and the Pacific Highway between Wardell and the Bruxner Highway intersection (11%). Remaining vehicle strikes were recorded from several minor roads elsewhere in the IPFA. None of the aforementioned roads have dedicated mitigation structures in place.

Figure 3.5 illustrates trends in the reported numbers of koalas being killed by vehicle-strike over the 26-year period to which the records relate, the number of known mortalities not appearing to have varied significantly over time (r^2 = 0.044, F = 0.7835, P = 0.3884). However, male koalas were nearly twice as likely to be the victim of vehicle-strike than were female koalas (Ratio: 1.80: 1.00; X^2 = 4.571, P = 0.033).

The <u>minimum</u> number of annual koala mortalities attributable to vehicle-strike is 2.00 ± 0.30 (SD) koalas annum^{-1}.

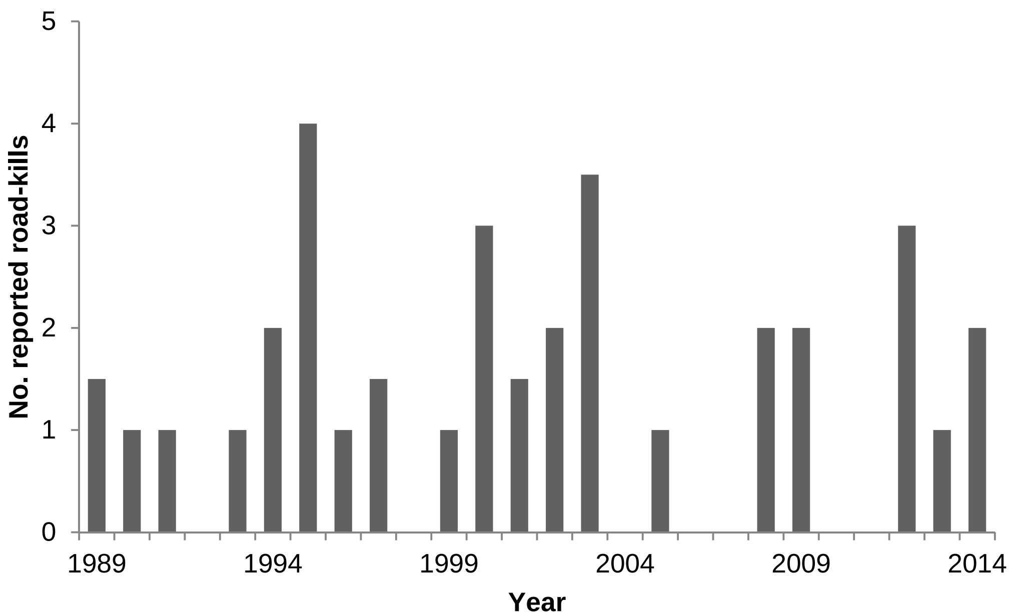


Figure 3.5 Numbers of known koala mortalities across the IPFA attributable to vehicle-strike over the time period 1989 – 2014.

Standardized in terms of the number of koalas kilometre^{-1} annum^{-1}, Table 3.2 further partitions vehicle-strike data to identify those roads within the IPFA where mortalities were most commonly reported. This approach identified the section of the Bruxner Highway to the east of Alstonville down to the intersection with the Pacific Highway as making the highest contribution to vehicle-strike mortalities within the IPFA (Table 3.2).

Table 3.2 Standardised koala vehicle-strike data for major roads within the IPFA. Numbers in brackets represent additional (i.e. non-FoK) records from NSW Wildlife Atlas that have been included in the “n” value being used. The values in the “Average” column were calculated by dividing “n” by the number of years (26) to which the data relate.

Road	*n*	Average	km	no. koalas km^{-1} $annum^{-1}$
Pacific Highway	7(3)	0.269	10.5	0.026
Bruxner Highway	8	0.308	5	0.062
Wardell Road	9.5(5)	0.365	9	0.041
Bagotville Road	7.5	0.288	8.5	0.034

Disease

Disease-related deaths were the single biggest contributor to koala mortality across the IPFA, responsible for approximately 36.7% ± 4.62% (SD) (n = 40/109) of all cause-known koala deaths between 1989 and 2014, the most commonly recorded cause being “Conjunctivitis/Cystitis”. Diseased koalas were most commonly reported from the Alstonville/Uralba and Meerschaum Vale localities (33% and 30% respectively).

Changes in the numbers of koalas known to have died from disease over the period 1989 – 2014 are illustrated in Figure 3.6 and arguably infer the presence of a cyclical phenomenon occurring across the IPFA landscape with peaks over 15 – 17 year time frames. Similar apparent cycles have been detected recently for the Richmond Valley Council area by Phillips and Weatherspoon (2015)[15]. It is noteworthy that the two peaks illustrated in Figure 3.6 do not arise in the same areas (and thus in a spatial sense are mutually exclusive), that for the period 1994 – 96 associated with the Meerschaum Vale and Wardell localities while that for 2007 – 2009 appears to be primarily associated with the localities of Coolgardie and Uralba. Equal numbers of male and female koalas were recorded as dying from disease.

[15] Koala Habitat & Population Assessment: Richmond Valley Council LGA. Final Report to Richmond Valley Council. Biolink Ecological Consultants, Uki NSW.

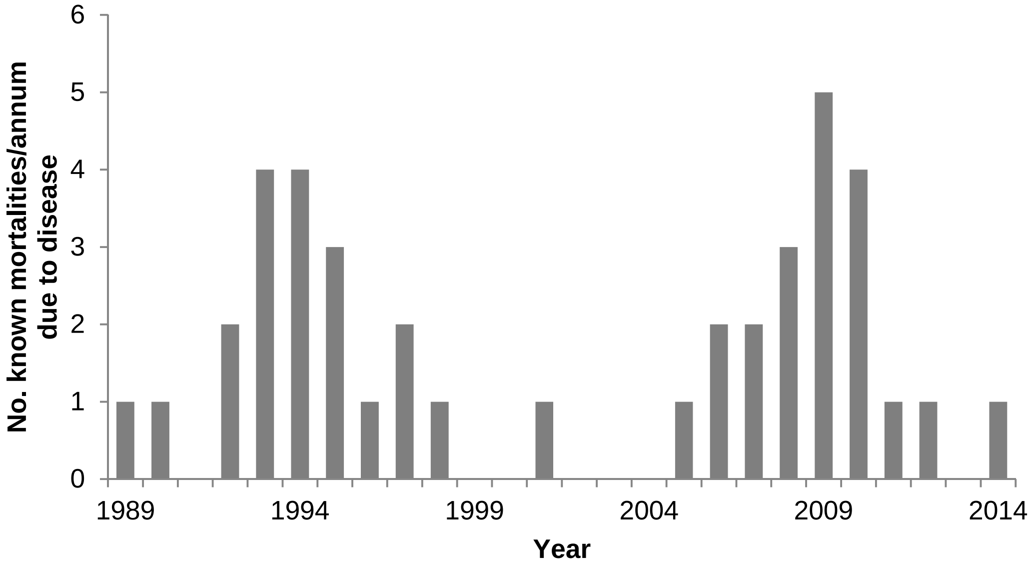


Figure 3.6 Numbers of known koala mortalities attributable to disease within the IPFA over the time period 1989 – 2014.

3.4 Key outcomes

Koala call-out data provided by FoK for the Ballina LGA was available for the 26-year time period between 1989 and 2014. The majority of koala call-outs and associated mortality data across the Ballina LGA were derived from the IPFA, *defacto* confirming the latter area as a significant population centre for koalas in the LGA.

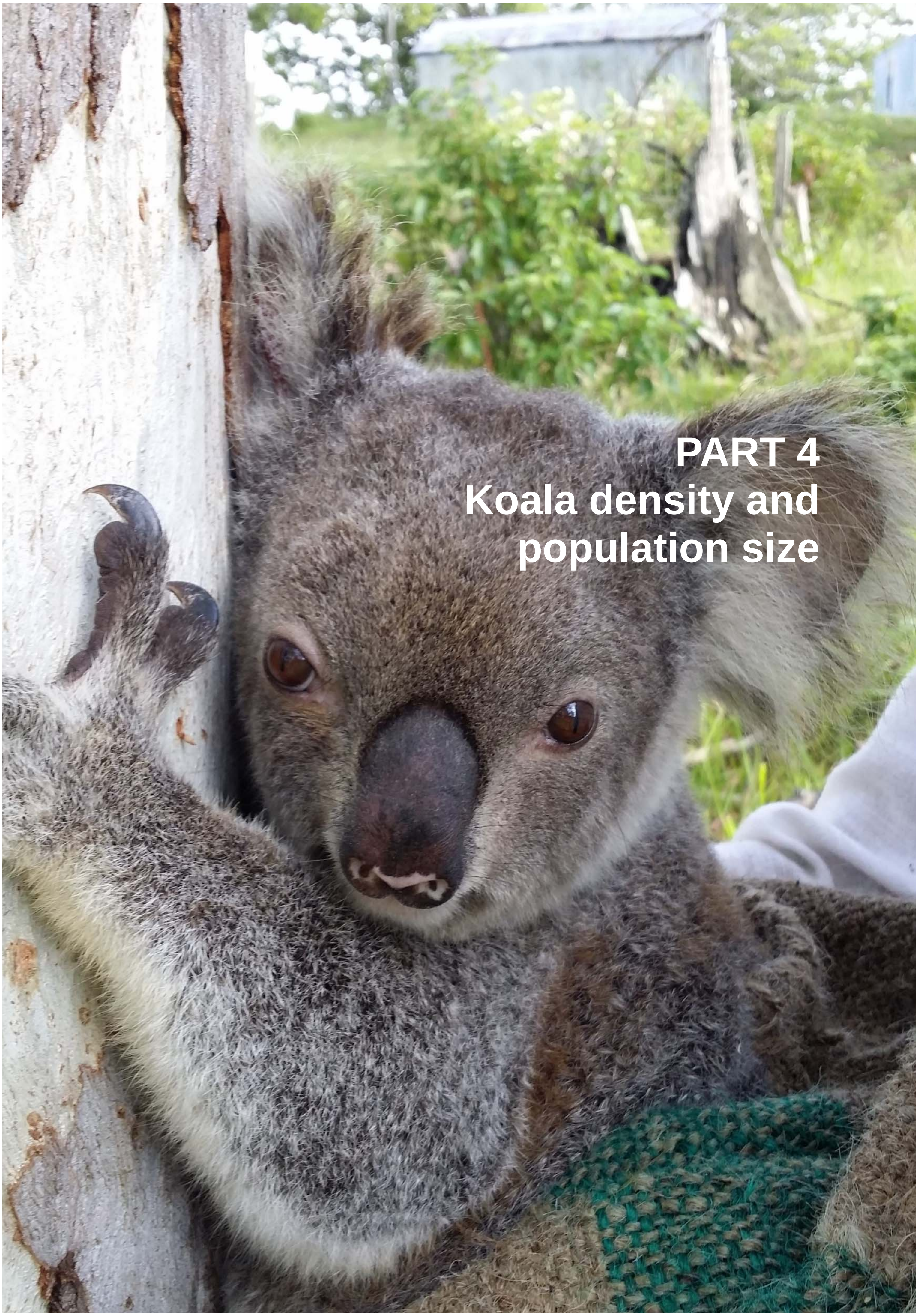
Incidental mortalities due to domestic dog attack and vehicle strike comprise more than 50% of all known koala mortalities on an annual basis. Concordant with the male-biased mortality earlier alluded to by the results obtained in Part 2 of this report, significantly greater numbers of male koalas are being killed within the IPFA by vehicle strike than are female koalas. The six koalas known to have been killed by vehicle-strike within the six-month period of the demographic study exceeds the maximum number of koalas known to have been killed annually by motor vehicles over the 26 year period to which the FoK records relate.

Changes in the numbers of koalas known to have died from disease over the period 1989 – 2014 implies the presence of a cyclical phenomenon occurring at a landscape scale with two peaks 15 – 17 years apart. Based on the information contained in this section, the peaks appear to be independent of climatic considerations such as low rainfall cycles, while levels of incidental harvest have remained relatively constant over the time period between the two peaks. In the absence of other data, this outcome potentially points to a largely unrecognized role of disease as a primary regulator of koala abundance across the IPFA. The extent to which anthropogenic-related causes such as habitat loss and/or modification may contribute to this mortality cycle remain speculative and problematical to differentiate.

Standardising the road-kill data clearly enables koala black spots to be better identified and (if required) prioritized for remedial action. The variable '*no. koalas killed km^{-1} annum^{-1}*' enables koala black spots to be prioritized for remediation while also having utility for PVA purposes by enabling adjustment of road-kill potential along areas affected by the conditionally approved Section 10 upgrade. Based on data collected from roads to the north and observations from this study, a multiplication factor of between 2 (minimum) and 3 (precautionary) would appear necessary if any meaningful estimation of the real numbers of koalas being killed annually by vehicle strike is required.

Key anthropogenic drivers of koala mortalities such as vehicle-strike are typically density-dependent harvesting mechanisms that operate at population level. While the downward trend in fatalities due to domestic dog attack was not statistically significant, it may also reflect an increasing absence of koalas in areas where domestic dog densities are high and dog ownership is long standing. In contrast, the lack of any significant change in the numbers of koalas being killed by vehicle strike over the 26 years to which the records relate may imply little change in the numbers of koalas inhabiting the IPFA over this period.

PART 4
Koala density and population size



4.1 Objective

To refine the previous koala density and population estimate arising from the BKS in order to derive a better understanding of the size of the koala population inhabiting the IPFA. This estimate provides a key input parameter (i.e. initial population size) for the PVA modelling process.

4.2 Methods

4.2.1 Site selection

Seventy-two locations within the IPFA were identified as potential field survey sites. Forty two of these sites were originally sampled for purposes of the BKS, while a further 30 higher resolution sites focused specifically on the proposed Section 10 alignment for the additional survey work reported by Ecosure (2014)[16]. Direct counts of koalas within a 25 m fixed radius (0.196 ha) at each field site had been undertaken as a component of the BKS survey program. This work provided an initial density estimate ($Ø_1$) of 0.12 ± 0.05 (SD) koalas ha^{-1} within the IPFA.

4.2.2 Field survey

The group of 72 potential survey sites was reduced to 59 by excluding sites located in areas of dry rainforest and camphor laurel. At these latter locations, direct counts of koalas were undertaken along transects that were 250 m in length and 40 m in width to cover a total area of approximately one ha. Direct counts of koalas at each transect site involved three observers walking ~ 20 m apart, one on the centre line and one on either side. Transects were generally oriented north-south (on flat to undulating terrain) or along the contour (on steeper terrain) and were generally commenced 125 m from the site co-ordinates, continuing for a further 125 m past this location.

4.2.3 Koala density/population estimate

Because of the larger area being searched when compared to the 25 m radius searches that were undertaken for purposes of the BKS, it was envisaged that a second and more refined koala density estimate ($Ø_2$) for the IPFA would be obtained by dividing the theoretically greater number of koalas counted during transect searches by the pooled search area of all transects. The resulting density data could then be extrapolated across the total area of preferred koala habitat (2,152 ha) mapped within the IPFA (see Part 1) in order to derive a population estimate.

[16] *Woolgoolga to Ballina koala preconstruction surveys - final report*, Report to Roads and Maritime Services, West Burleigh

4.3 Results

4.3.1 Survey effort

Forty-six field sites were surveyed between 13 April and 24 April 2015 covering a total search area of 45.34 ha. Thirteen of the 59 targeted sites could not be accessed due to either inundation of low-lying coastal sections of the IPFA or retraction of earlier consents from land owners on whose properties the sites were located.

4.3.2 Koala sightings

Eleven koalas were sighted during the course of the surveys, three of which were recorded within the 45.34 ha covered by the transect searches, the remainder elsewhere within the IPFA while traversing between survey sites. Three of the koalas that were observed, including one from the transect surveys, had been previously captured as part of the demographic profiling component of this project (Part 2 refers).

The distribution of survey sites and the location of koala sightings are illustrated in Figure 4. 1. with individual site coordinates and further details provided in Appendix 2

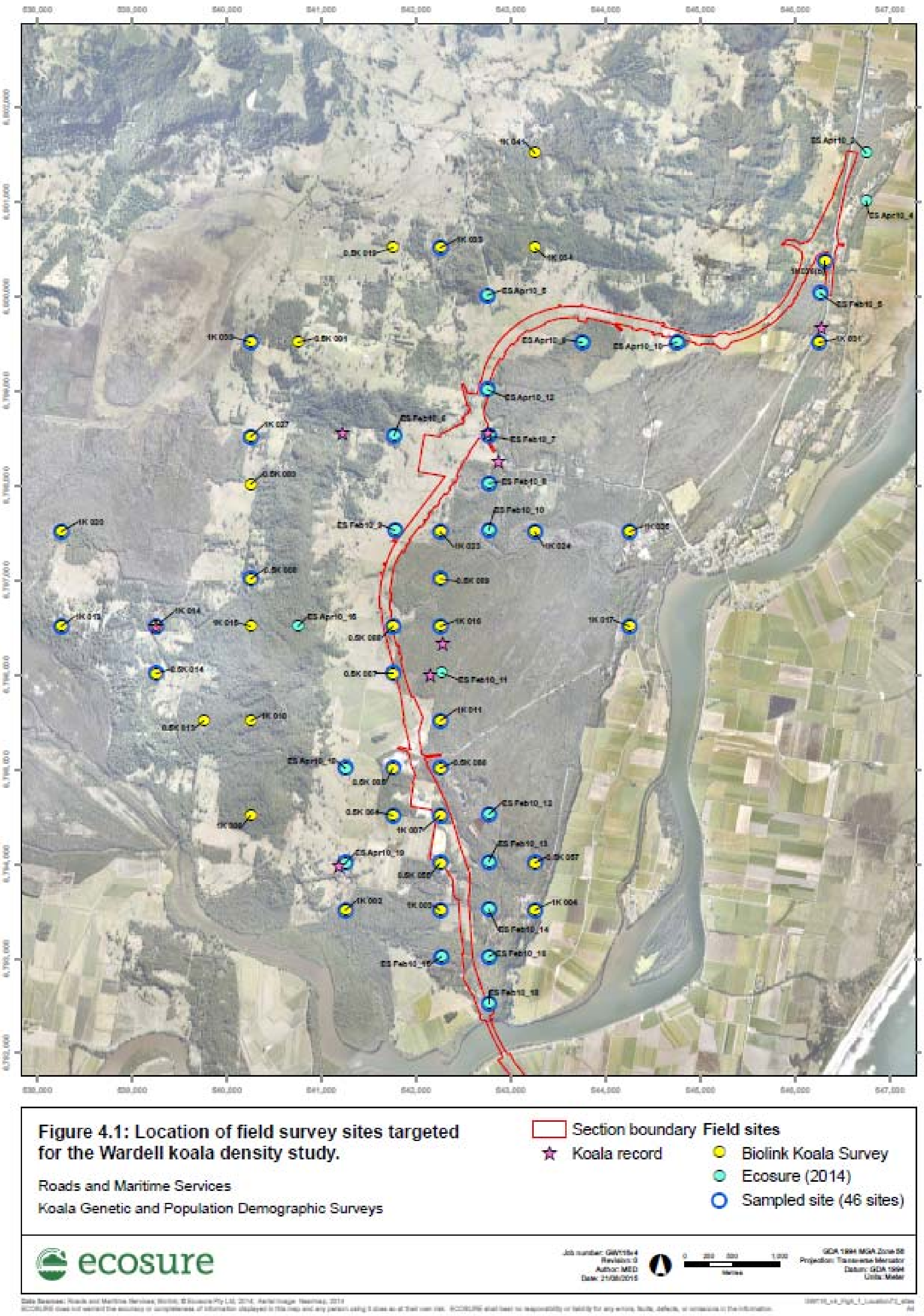


Figure 4.1 Location of 72 field survey sites targeted for the Wardell koala density study. Blue sites represent sites from Ecosure (2014) and yellow represent sites from the BKS.

4.3.3 Koala density/population estimate

Based on the presence of three koalas within the 45.34 ha covered by the transect searches, koala density ($Ø_2$) was estimated at 0.066 ± 0.037 (SD) koalas ha^{-1}. When this density estimate was extrapolated across the 2,152 ha of preferred koala habitat within the IPFA a population estimate of 142 ± 80 (SD) koalas resulted which was lower than the original estimate of 259 ± 107 (SD) derived from the BKS data. In order to lessen the uncertainty around these estimates and guided by the work of Roberts and Binder (2009)[17] we pooled survey outcomes arising from the BKS field surveys with those of this survey to obtain a refined density estimate ($Ø_3$) as follows:

$$Ø_3 = (n_1Ø_1 + n_2Ø_2)/(n_1 + n_2) \qquad \text{(Eqn 3)}$$

Where:

$Ø_3$ = refined koala density estimate

$Ø_1$ = density estimate from previous work; 0.12 ± 0.05 (SD) koalas ha^{-1}

$Ø_2$ = density estimate from this study; 0.07 ± 0.04 (SD) koalas ha^{-1}

n_1 = number of survey sites associated with $Ø_1$

n_2 = number of survey sites associated with $Ø_2$

with a Standard Deviation of:

$$\sigma = \sqrt{}(Ø_{3*}(1-Ø_3)/(n_1 -1 + n_2 -1) \qquad \text{(Eqn 4)}$$

Where:

σ = standard deviation of the refined estimate

$Ø_3$ = refined koala density estimate

n_1 = number of survey sites associated with $Ø_1$

n_2 = number of survey sites associated with $Ø_2$

This approach yielded a refined density estimate of 0.091 ± 0.03 (SD) koalas ha^{-1} and a corresponding population estimate for the IPFA of 196 ± 65 (SD) koalas

[17] Analyses based on combining similar information from multiple surveys. *Journal of Statistical Mechanics*. 2138 – 2147.

4.4 Key outcomes

Previous survey work undertaken for the BKS by way of 25 m fixed radius searches for koalas at 42 field sites across the IPFA yielded a koala density estimate of 0.12 ± 0.05 (SD) koalas ha^{-1} and a consequent population estimate of 259 ± 107 (SD) koalas.

Direct counts of koala numbers were undertaken in 46 x 1 ha transects uniformly distributed across the IPFA. Eleven koalas were sighted, three of which were recorded in the 45.34 ha covered by the transect searches to provide a density estimate of 0.07 koalas ha^{-1}, with a corresponding population estimate for the IPFA of 142 koalas. Three of the 11 koalas that were sighted during the survey program had previously been captured for purposes of the demographic study reported elsewhere (Part 2 refers).

Despite a low koala encounter rate in both survey events, pooling of survey results from both the BKS and this assessment enabled a more refined density estimate to be derived resulting in a population estimate of 196 ± 65 (SD) koalas across the IPFA.

PART 5
Koala metapopulation distribution east of the proposed section 10 upgrade

5.1 Objectives

Accurately determining the distribution of koala populations across a given landscape is essential for koala population conservation and management planning. This component of the overall project was focussed on the following objectives:

(i) To further refine/improve knowledge about the location and extent of areas currently occupied by resident koala populations to the east of the conditionally-approved highway alignment; and

(ii) To identify areas of suitable habitat that are currently unoccupied or that appear to currently support transient use by koalas that may be suitable for consideration as potential translocation sites if required.

5.2 Methods

5.2.1 Survey area

The survey was restricted to an area of approximately 1,750 ha north of the Richmond River, west of the existing Pacific Highway alignment and associated urban environment of Wardell and east of the conditionally-approved alignment for the Section 10 upgrade (Figure 1 refers). With the exception of some elevated lands in the southwest, the majority of koala habitat in the survey area is comprised of low-lying swamp and dry sclerophyll woodlands and forests and heathlands on coastal sandplains and alluvial deposits. As detailed by the BKS, koala habitat in the lower-lying areas is determined by the localised presence of swamp mahogany *E. robusta*, forest red gum *E. tereticornis* and the naturally-occurring hybrid between these two species (sometimes referred to as *E. patentinervis*) while in more elevated sections of the survey area, tallowwood *E. microcorys* is typically the most important koala food tree species.

5.2.2 Survey design

A 350 m point-based grid overlay anchored to the original 500 m BKS grid was initially utilised to sample koala activity/density, with primary field sites located wherever grid intersections occurred in areas of native vegetation containing eucalypts. Supplementary field sites were subsequently included at intermediate intervals between high or medium use and low use sites[18] in order to further inform the modelling process by more precisely delineating areas of significant koala activity. Coordinates for each site were uploaded into hand-held GPS receivers (GDA94 datum) in order to assist navigation in the field. Where necessary the precise location of sites was adjusted in the field to avoid areas of temporary inundation and/or to incorporate eucalypts within the sample. Landowners were contacted to seek permission before entering private properties that contained survey sites.

5.2.3 Site assessment

Once located, each site was sampled using the Spot Assessment Technique (SAT) of Phillips and Callaghan (2011)[19]. A default high use activity level was applied as soon as ten trees scored positive for koala faecal pellets at any site, thus surpassing the minimum threshold for classification as high use. Conversely, if the first 25 trees scored negative for faecal pellets, a default low use activity level was applied as this outcome would not change regardless of the score for the remaining five trees at the site.

In accordance with the SAT, the results of field survey are described in terms of 'active sites' and 'koala activity'. An active site is any site where one or more koala faecal pellets were recorded in the search catchment around the base of one or more of the sampled trees, while the associated koala activity level is calculated as the percentage of surveyed trees that

[18] As defined by: Phillips, S. and Callaghan, J. 2011.

[19] The Spot Assessment Technique: a tool for determining localised levels of habitat use by Koalas. *Australian Zoologist*. **35(3)**: 774-80.

scored positive for the presence of koala faecal pellets (Eqn 5 refers).

$$\text{Activity Level} = \frac{\text{Number of trees with faecal pellets}}{\text{Number of surveyed trees}} \times 100 \qquad \text{(Eqn 5)}$$

5.2.4 Koala activity modelling

Koala activity data from all sampled field sites were interpolated across the study area using regularised, thin-plate splining techniques through the spatial analyst extension in ArcGIS 9.3. To assist this process null points were incorporated into the modelling process at regular intervals (~ 100 m) in order to (i) delineate major distributional and/or dispersal barriers such as the Richmond River, and (ii) constrain the modelling process to areas east of the conditionally-approved highway alignment. The general approach is explained in more detail within Phillips *et al.* (submitted)[20].

The output from the splining process was then utilised to produce an activity contour model to delineate areas occupied by resident koala populations by identifying those contours concordant with the 10%[21], 23% and 33% significant activity thresholds of Phillips and Callaghan (2011) as detailed in Table 5.1. Additional contours were included in the activity model as required to assist with interpretation of connectivity.

Table 5.1 Categorisation of koala activity based on use of mean activity level ± 99% confidence intervals for each of three area/population density categories. Activity levels in the Medium (normal) and High use range indicates occupancy by resident koala populations (Source: modified from Phillips and Callaghan 2011).

Activity Category	Low use	Medium (normal) use	High use
Area (koala density)			
East Coast (low)	-	≥ 9.99% but ≤ 12.59%	> 12.59%
East Coast (med-high)	< 22.52%	≥ 22.52% but ≤ 32.84%	> 32.84%

5.2.5 Koala density estimates

Independent of the transect searches reported in Part 4, a 25 m fixed-radius (0.196 ha) search for koalas was undertaken around the centre tree at each SAT survey site irrespective of the resulting activity level. A koala density estimate could then be derived by dividing the total number of koalas recorded in the 25 m fixed-radius searches associated with each primary field site, by the sum of the areas searched using this method. Supplementary field sites were not included in this assessment.

[20] Modelling koala population structure across the landscape in order to provide greater certainty for conservation and management purposes. *Landscape and Urban Planning.*

[21] Applies only to low nutrient elevated sites in the extreme south of the survey area.

5.3 Results

5.3.1 Koala activity

Surveys were undertaken between 27 April and 22 June 2015, during which time 53 primary and 23 supplementary field sites were assessed. Preferred koala food trees were present in 22 of the 53 primary field sites that were sampled (41.51%).

Evidence of koala activity (i.e. koala faecal pellets recorded beneath at least one tree within a given field site) was detected at approximately 55% (29/53) of the primary field sites wherein koala activity levels ranged from 3.33% to 100% (mean activity level (active sites only): 19.42% ± 7.48% [SD]). Nine of the primary field sites returned significant (i.e. medium or high) koala activity levels (i.e. >22.52% activity for med-high population density sites on coastal lowlands, plus one >9.99% low-density site on elevated erosional soils in the south) consistent with the presence of resident koala populations. When considered in the context of the total number of primary field sites that contained preferred koala food trees (*n* = 22) these data enable an occupancy estimate of 40.91% ± 10.73% (SD) across the area covered by the survey program. As with the density survey detailed in Part 4 of this report, heavy rainfall and inundation of low-lying areas in the week preceding the commencement of surveys impeded access to a number of low-lying, swamp forest sites in central parts of the survey area.

The distribution of survey effort across the survey area is illustrated in Figure 5.1, while the associated site coordinates and resulting activity levels are detailed in Appendix 3.

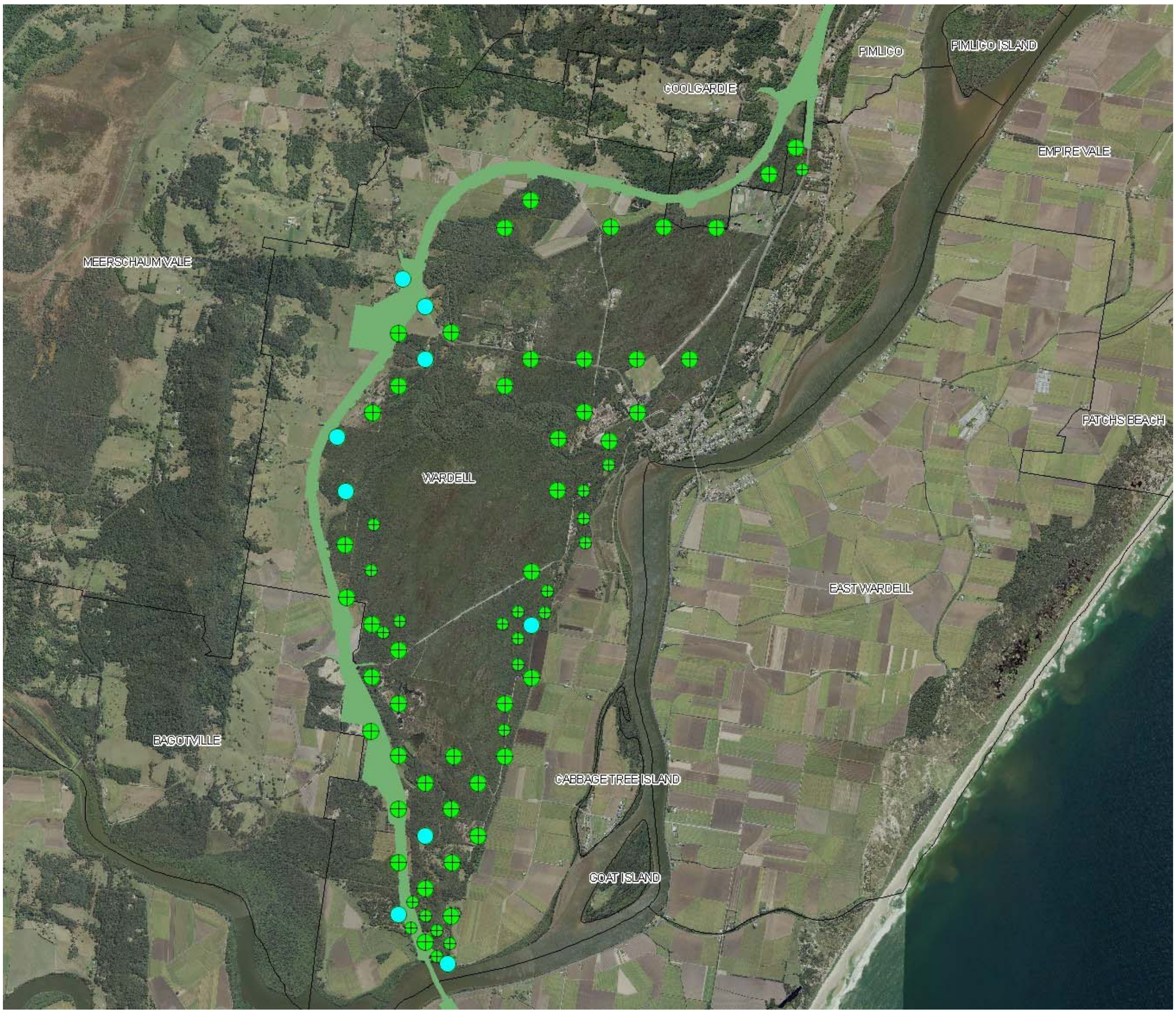


Figure 5.1 Location of 53 field survey sites used for the Wardell koala meta-population study. Surveyed primary field sites are shown as large green dots, those that returned significant activity levels highlighted in blue, supplementary sites are shown as small green dots

5.3.2 Koala density

One koala was recorded within the 10.39 ha of habitat collectively sampled by the 53 x 25 m radial koala searches undertaken at primary field sites. This data translates to a koala density estimate within the area surveyed by this study of approximately 0.096 ± 0.08 (SD) koalas ha^{-1}.

5.3.3 Activity modelling

Koala activity was discretely clustered around suitable habitat areas predominantly located around the periphery of the survey area. The resulting koala population distribution model based on the koala activity is provided in Figure 5.2.

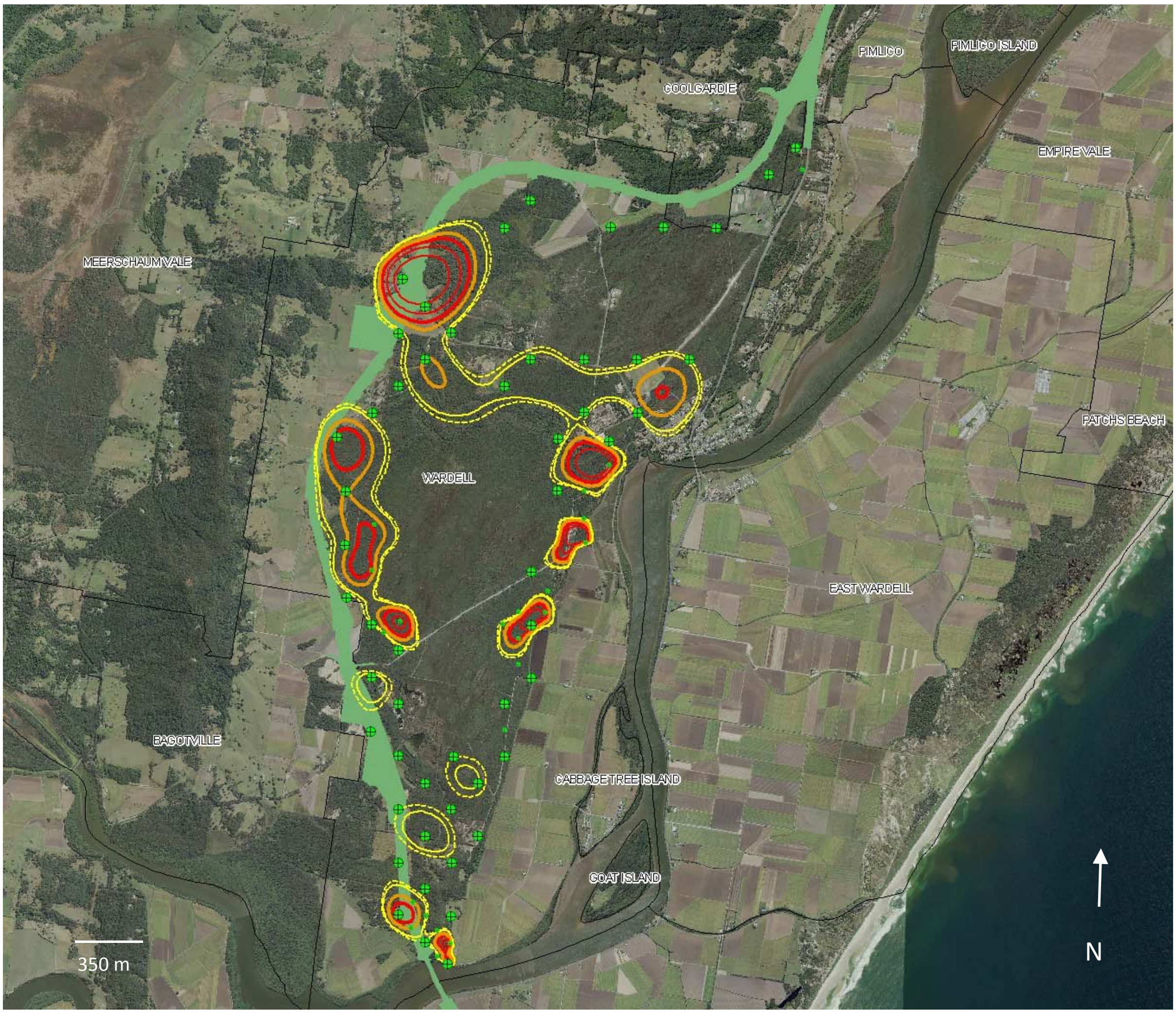


Figure 5.2 Koala activity model for the Wardell meta-population study area. Surveyed primary field sites are shown as large green dots, supplementary sites are shown as small green dots. Dotted yellow lines represent the 7% activity contour, solid yellow lines = 10% activity, thick orange lines = 23% activity (i.e. medium use), thick red lines = 33% activity (i.e. high use), additional narrow red lines = 50% and 75% activity respectively.

The model identifies cells of significant (i.e. medium to high) koala activity in the central west, northwest, northeast and central east, and far south sections of the surveyed area. These cells generally coincide with locations supporting vegetation types that include preferred koala food tree species and also coincide with locations where the majority of koala sightings were recorded during the field survey program. The localities where the cells have been identified are shown in Figure 5.2 and are described briefly as follows:

- Locality 1 (Bingle Creek) – areas with scattered clumps of swamp mahogany woodland associated with drainage lines amongst shrubland communities on Jali lands on the western fringe of Wardell. This cell is ~ 19 ha in size.
- Locality 2 (Bingle Creek) – swamp mahogany and forest red gum dominated forest and woodland areas on Jali lands adjoining Bingle Creek near the southwestern edge of Wardell. This cell is ~ 21 ha in size.
- Locality 3 (Old Bagotville Road East) – swamp mahogany and forest red gum dominated forest and woodland areas on Jali lands that adjoin cane farming lands to the southeast. This cell is ~ 11 ha in size.

- Locality 4 (Old Bagotville Road South) – swamp mahogany dominated forest and woodland areas on Jali lands to the south of Bagotville Road, extending along the interface with the cane farming lands to the east. This cell is ~ 8 ha in size.
- Locality 5 (Back Channel Road) – forest and woodland areas containing forest red gum and tallowwood on foot-slopes and low ridges on private property in the southern extent of the study area, running northwards from the Richmond River. This locality consists of two linked cells totaling ~ 20 ha in size that will be directly impacted by the conditionally-approved upgrade. Based on the estimated carrying capacity for secondary (class B) habitat (as detailed in Table 1.1 in Part 1) of ~ 0.23 koalas ha^{-1} multiplied by the overall size of the cell (~ 20 ha) and allowing for a difference of one koala either side of the product, we estimate the number of koalas to be impacted in this area as four to six individuals.
- Locality 6 (Western Jali Lands) – forest and woodland areas containing swamp mahogany. This locality comprises three linked cells totaling ~ 80 ha in size, the northern-most of which is peripherally impacted by the upgrade alignment. Based on the carrying capacity for secondary (class A) habitat (as detailed in Table 1.1 in Part 1) of ~ 0.42 koala ha^{-1} multiplied by a disturbance area of ~ 3 ha, we estimate the number of koalas to be impacted by clearing activities in this area as likely to be no more than two individuals.
- Locality 7 (Thurgates Lane to Buckombil) – slightly elevated forest and woodland areas containing tallowwood and low-lying areas containing forest red gum on private property in the eastern section of Thurgates Lane extending across Wardell Road to the north. This locality comprises two linked cells totaling ~ 60 ha in size that will be directly impacted by the conditionally-approved upgrade. Based on the carrying capacity of secondary (class A) habitat (as detailed in Table 1.1 in Part 1) of ~ 0.42 koalas ha^{-1} multiplied by an estimated area of direct impact associated with the road alignment of ~ 10 ha, we estimate the number of koalas to be impacted in this area as four to six individuals.

The preceding outcomes remain qualified by the fact that we were unable to access all areas due to heavy rainfall and inundation of some low-lying swamp forest sites. Because of this we cannot exclude the possible occurrence of additional activity cells within the swamp forest areas to the immediate south of Thurgates Lane, Wardell Road and Bingle Creek generally. Regardless, when overlain with koala habitat mapping and subject to the aforementioned qualification, the model enables identification of two relatively large and currently unoccupied or under-utilised areas of preferred koala habitat (see Figure 5.3) as follows:

- Site 1 (Central Jali lands) - areas dominated by swamp mahogany extending southwards between occupied localities 3 (Old Bagotville Road South) and 4 (Back Channel Road) in the south-eastern section of the study area. This site is bordered in the west by dry heathland and woodlands that are largely devoid of preferred koala food trees with the exception of dune swale areas, but which support supplementary food and shelter resources. This site is ~ 30 ha in area and is estimated to be potentially capable of sustaining a small population cell of up to ~ 12 additional koalas.

- Site 2 (Lumley's Lane Jali Lands and adjoining areas of private property) – areas dominated or co-dominated by swamp mahogany in the north of the study area off Lumley's Lane to the south and between Lumley's Lane and the current Pacific Highway alignment. This site consists of fringing habitat patches bordered primarily by eucalypt woodlands, swamp forests, heathlands and shrublands on sandy substrates. It is estimated that these sites would collectively total ~ 15 to 20 ha and could potentially sustain ~ six to nine additional koalas.

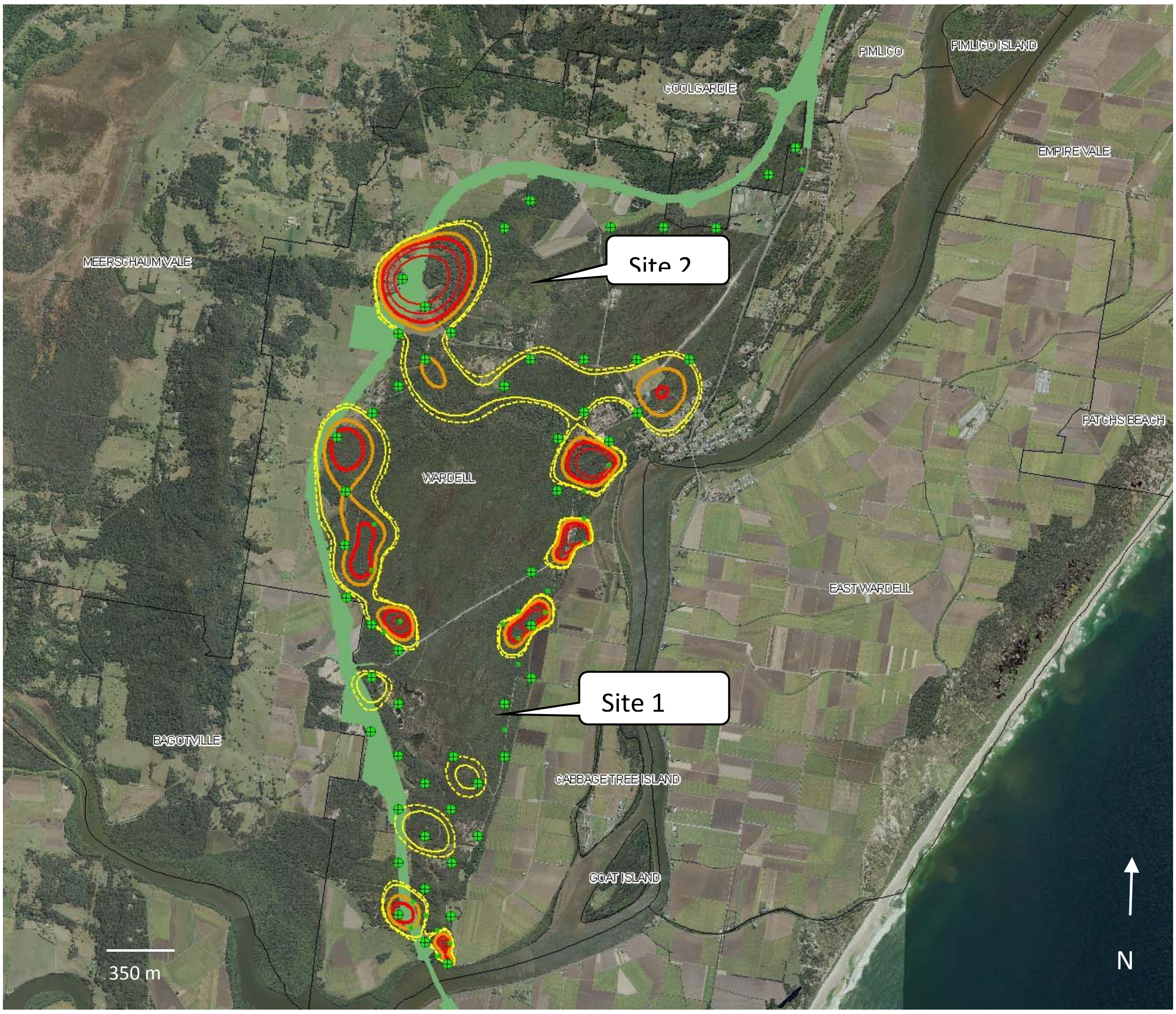


Figure 5.3 Locations of currently unoccupied and/or under-utilised areas of high carrying capacity preferred koala habitat.

5.4 Key outcomes

SAT surveys were undertaken at a total of 76 sites (53 primary sites and 23 supplementary sites) across forested parts of the study area to the east of the conditionally-approved Section 10 highway alignment.

The potential impacts on the local koala population associated with the conditionally-approved Section 10 upgrade are accentuated by the focus of koala activity and distribution in areas that overlap or adjoin preferred koala habitat in the west.

Whilst the activity model was confined to the area to the east of the conditionally-approved Section 10 alignment, it is apparent that koala activity links across the upgrade alignment at two localities, the most important of which occurs at Locality 7 (Thurgate’s Lane to Buckombil Mountain Road). In contrast, and informed by the field surveys undertaken for this Part, there is no evidence of continuity of significant koala activity in the vicinity of Chainage 147600, a locality previously identified for a fauna land bridge.

A density estimate of approximately 0.096 ± 0.08 (SD) koalas ha^{-1} was derived for suitable habitat within the meta-population study area. This density estimate is in accord with that estimated by the BKS and the transect surveys detailed in Part 4 of this report.

Unless properly managed, it is estimated that the impact of constructing the conditionally-approved Section 10 highway alignment has the potential to result in the displacement of ~ 10 to 14 koalas.

Two areas (Figure 5.3) containing preferred koala habitat were identified by the metapopulation study with expected high carrying capacity that do not appear to currently support resident koalas.

The activity model can be overlain with the road footprint for an enhanced understanding of potential future impacts upon the resident koala population as a result of the conditionally-approved highway alignment and associated changes to local road traffic volumes.

PART 6
Population status & derived PVA baseline input parameters

6.1 Objective

The purpose of this Part is to detail specific recommendations arising from Parts 1 – 5 of this report insofar as they relate to the baseline input parameters required to inform the PVA process.

6.2 Population status

The preceding parts of this report have revealed the presence of a relatively small and widely dispersed koala population. Based on the average annual mortality rate of approximately 10% and the information detailed in Parts 2 and 3, incidental mortalities due to vehicle-strike and attacks by domestic dogs account for more than half of all koala deaths annually. Because of this, striving to increase population size and/or carrying capacity will arguably be ineffective if incidental mortality rates cannot be reduced. Indeed, based on the premise that the mortalities due to domestic dog attack and vehicle-strike are the primary drivers of population decline in the population, we speculate that a reduction in the cohort-based mortality rates of approximately 50% will likely be required in order to place the population on a more sustainable footing.

6.3 Parameters of acceptable impact

In approving the W2B Pacific Highway upgrade and with particular regard to koalas occupying the IPFA, amongst other matters, the following condition was specified:

"*The (Federal Environment) Minister will only approve the plan and the commencement of Section 10 of the action if the impacts to the Ballina Koala population are demonstrated to be acceptable within the Ballina Koala Plan*."

The authors propose the following principle as a basis for addressing this key condition through the PVA and koala planning process:

"The PVA-modelled measures of population status (e.g. rate of decline, mean population size) and the associated Probability of Extinction (PE) of the koala population inhabiting the IPFA in a post-construction Section 10 highway upgrade scenario, must at the very least not be worsened but ideally improved from the pre-construction baseline PVA-modelled outputs."

6.4 Baseline input parameters

(i) Single or multiple populations?

The BKS identified four clusters of koala activity within the IPFA. These four clusters have been loosely referred to as the eastern and western cells, the former relating to areas occupied by resident koala populations of the Ngunya Jargoon IPA and adjoining localities, the western cell comprising populations extending northwards from Bagotville through Meerschaum Vale and Buckombil Mountain and thereafter northwards to Coolgardie – Uralba. Based on knowledge gained during the work detailed herein, we consider the western and eastern population cells in the current landscape configuration to be demonstrably in contact in two localities. Thus for baseline PVA modelling purposes, but conditional upon results from the genetic analysis, we propose that the IPFA population be regarded as a **single** population.

Based on the requirements of Vortex Version 9.99 the following baseline input parameters are proposed:

(ii) Scenario settings

We propose a minimum of **500** iterations over a **50-year** time frame with extinction defined as only **one sex remaining**.

(iii) Species description

Inbreeding depression

Based on the observations reported in Part 2 (Demographic Profile) but subject to additional information that might be forthcoming from the genetic study, we do not consider that any potential exists that would warrant inbreeding depression being incorporated for baseline PVA modelling purposes.

Environmental Variance (EV) concordance

We propose that EV concordance be confirmed.

Number of types of Catastrophes

Information supplied in Part 1 (Introduction) supports the incorporation of **two** potential catastrophes as baseline PVA input parameters (further details are provided in section *vii* below).

(iv) Dispersal among populations

Not required for baseline PVA input parameter purposes.

(v) Reproductive system

The koala's reproductive system/life history strategy is **polygynous**.

- Age of first offspring for Females: **2**
- Age of first offspring for Males: **4**
- Maximum age of reproduction: **8**[22]
- Maximum number of broods year^{-1}: **1**
- Maximum number of progeny brood^{-1}: **1**
- Sex ratio at birth in % males*:* **50**

Density Dependent Reproduction

Should this data be required for subsequent PVA modelling but otherwise based on data in Part 2 (Population demographics) we estimate the percent of females to be reproducing at low density **P(0)** to be ~ **65%** and the percent of females to be reproducing at high density **P(K)** to be ~ **30%**.

(vi) Reproductive rates

% Adult females breeding

Based on the information provided in Part 2 (Population demographics) we estimate a value of **44.83% ± 9.27% (SD)** as indicative of the numbers of adult females breeding on an annual basis.

Distribution of broods per year

As applicable to a single population.

Specify the distribution of number of offspring per female per brood

As applicable to a single population.

(vii) Mortality Rates

Based on the outcomes arising from Part 2 (Population Demographics), we advise the following baseline mortality rates as detailed in Table 6. 1, the associated Deviations due to Environmental Variation (D/EVs) estimated on the basis of the Coefficient of Variation of **59.06%** determined in Part 3 (FoK Mortality Data) as applying to the mean number of dead koalas being reported annually across the IPFA. For PVA purposes the percentage representation of the number of koalas in each TWC cohort as a function of estimated population size (P%) is also provided.

[22] Based on estimated maximum age of a breeding female koala in TWC 5 from this population (Table 2 in Gordon 1991 refers).

Table 6.1 Estimated baseline mortality rates (M) and associated standard deviations due to environmental variation for the IPFA koala population. The column "P" details the estimated percentage representation of each cohort as currently represented in the population and should be used to calculate numbers for purposes of detailing specific age distribution for PVA purposes based on current (2015) population estimate of 196 koalas.

Age (years)	TWC	TWS	P(%)	M(%)	SD_{EV}
Females					
1	1	P4A	-	19.7	11.63
2	2	P4B	6.00	19.7	11.63
3	3	P4C	12.00	26.21	15.48
4	4	P4D	9.00	7.34	4.33
5		P4D	9.00	7.34	4.33
6	5	P4E	9.00	5.37	3.17
7		P4E	9.00	5.37	3.17
8	6	P4F	3.33	2.76	1.63
9		P4F	3.33	2.76	1.63
10		P4F	3.33	2.76	1.63
Males					
1	1	P4A	-	19.45	11.49
2	2	P4B	4.00	19.45	11.49
3	3	P4C	2.00	30.56	18.05
4	4	P4D	10.00	4.3	2.54
5		P4D	10.00	4.3	2.54
6	5	P4E	3.00	6.29	3.71
7		P4E	3.00	6.29	3.71
8	6	P4F	1.33	2.92	1.73
9		P4F	1.33	2.92	1.73
10		P4F	1.33	2.92	1.73
			(~100)		

(viii) Catastrophes

The primary catastrophes to be incorporated as baseline input parameters are **drought** and **fire**. We assess the risk of these catastrophes for PVA purposes as follows:

Drought

Based on climate data provided in Part 1 (Introduction) we assess the probability of a period of "serious" to "severe"[23] rainfall deficiencies leading to a drought event within the IPFA to be three in every 14 years (i.e. frequency of ~ **0.21**), the potential extent of any event to be global but otherwise restricted to ridgeline habitat areas we have estimated to approximate 700 ha of the 2152 ha (i.e. **32%**) within which the impacts on reproduction and survival for PVE

[23] As defined by Australian Bureau of Meteorology

purposes are estimated to be **0.85** and **1.0** respectively.

Fire

Based on the fire history provided in Part 1 (Introduction), we assess the probability of a catastrophic fire event within the IPFA to be one in every 35 years (i.e. a frequency of ~ **0.03**) and we estimate the likely geographic extent of such an event to encompass ~ **10%** of the study area, with impacts on reproduction and survival likely to be severe to moderate, the proportional estimates for PVA purposes of **0.85** and **0.60** respectively.

(ix) Mate monopolization

As detailed in Part 2 (Population demographics) we propose that this measure equates to the estimated number of male koalas in TWCs 4 – 5, and therefore should be **76.47% ± 10.6% (SD)**.

(x) Initial population size

On the basis of data detailed in Part 4 (Koala density/population size) we advise a population size estimate for the number of koalas in TWCs 2 - 7 within the IPFA of **196 ± 65 (SD) koalas**. Based on the skewed sex ratios detailed in Part 2 of this report this number translates to a population currently comprised of **125** female and **71** male koalas.

(xi) Carrying capacity

Carrying capacity

On the basis of information provided in this report, we have calculated an upper limit estimate of 556 koalas for the IPFA, which could only apply if all available habitat were to be fully occupied. However, findings from other coastal populations support an expected population occupancy rate of slightly greater than 50% of available habitat. Hence, we propose that the koala carrying capacity **(K)** for baseline PVA modeling purposes be set at **291 ± 15 (SD) koalas.**

Future change in K

For reasons outlined in the Additional Notes below we propose that this parameter be left unchecked **(i.e. a zero change in K)** for the purposes of baseline PVA modelling purposes.

(xii) Harvest

No harvest is envisaged for the purposes of baseline input parameters.

(xiii) Supplementation

No population supplementation program currently exists for baseline input parameters.

(xiv) Genetic management

No genetic management program currently exists for the purposes of baseline input parameters.

6.5 Additional notes

Given that the conditionally-approved Section 10 upgrade traverses the south-eastern corner of the IPFA, we envisage that a range of management scenarios may need to be considered and possibly informed by a two- or even three-way metapopulation PVA model. To assist this process should it be necessary, some elements of the baseline input parameters may be further partitioned as follows:

6.5.1 Modelling two populations

If required for subsequent PVA scenario testing purposes, the population can be partitioned in terms of the numbers of koalas occupying habitat to the west and east of the proposed alignment as follows: **76.19% (149 koalas) in the west** and **23.81% (47 koalas)** in the east (Source of primary partitioning parameters: numbers of occupied field sites reported by the BKHS).

6.5.2 Modelling three populations

If required for subsequent PVA scenario testing purposes, the population can be partitioned in terms of the numbers of koalas occupying habitat to the north, west and east of the proposed alignment as follows: **19.05% (37 koalas) in the north**, **57.14% (112 koalas) in the west**, **and 23.81% (47 koalas) in the east** (Source of primary partitioning parameters: numbers of occupied primary field sites reported by the BKHS).

6.5.3 Future changes in K

It is acknowledged that approximately 130 ha of additional habitat is proposed to be re-created should the project proceed, this value will need to be reduced by the total area of habitat that would be lost to the road construction process and will need to factor in the time required for trees to advance to a stage where they could support koalas. Scenario modelling will also need to accommodate both the loss of preferred koala food trees as a consequence of Private Native Forestry practices (three licenses currently in force, one application currently being processed), and the ongoing removal (without replacement) of tallowwood windrows elsewhere within the IPFA.

6.5.4 Harvesting and associated mortality considerations arising from road construction

Construction of the conditionally approved Section 10 highway upgrade would result in a loss of vegetation cover, some of which currently supports resident koala populations. As detailed in Part 5 of this report (Wardell meta-population survey), three key localities have been confirmed where animals will be directly impacted by habitat modification/removal. These localities and the approximate number of koalas to be impacted in each instance are as follows:

1. Chainage 146000 – 146800 (Law's Point): displacement/disruption of resident koalas (*n* = 4 – 6).
2. Chainage 149600 – 151400 (Jali Lands (west): displacement/disruption of resident koalas (*n* = 2).
3. Chainage 152200 – 153600 (Thurgate's Lane to Buckombil Mountain Road): displacement/disruption of resident koalas (*n* = 4 – 6).

An appropriate strategy for management of these displaced koalas will be required for subsequent PVA modelling purposes, in the absence of which we would estimate that a minimum of 10 – 14 koalas must be assessed as potentially lost from the population as a consequence. The removal of these koalas from the population would need to be factored into the PVA model as a progressive harvest initiated at the date construction work commences and ending on the date construction works are completed.

Appendix 1 Koala capture data

Codes as follows: DoC = Date of Capture; FRS = Female Reproductive Score (Part 2 refers); TWS = Tooth-wear Score (Gordon 1991); COH = Cohort (A = Adult, S/A=Sub-adult); MCHIP = Microchip number; Other ID = identification assigned on admission to the FoK Care Centre or Currumbin Sanctuary Wildlife Hospital (CSWH), or the existing ear tag number

DoC	Easting	Northing	Sex	FRS	Weight (kg)	TWS	COH	Tag	MCHIP	Other ID	Detail
2/12/2014	543060	6806472	F	1	4.00	P4D	A	na	na	021214_01	Domestic dog attack
15/12/2014	541534	6793953	M	na	7.00	P4D	A	0381Red	76906CE	na	capture
16/12/2014	540292	6793889	F	2	5.4	P4E	A	0382Red	7690493	na	capture
18/12/2014	546412	6800737	F	1	4.9	P4C	A	na	na	181214_01	road-kill
18/12/2014	539495	6794281	M	na	6.1	P4D	A	0368Red	7710682	na	capture
2/01/2015	539639	6794338	M	na	6.9	P4D	A	0383Red	768FD69	na	capture
5/01/2015	545432	6805736	F	1	5.4	P4FM1G	A	0370Red	7693984	na	capture
7/01/2015	539678	6794086	F	4	5.7	P4E	A	0384Red	768F7DF	na	capture
8/01/2015	544274	6796413	M	na	7	P4D	A	0386Red	769143C	na	capture
8/01/2015	544290	6796262	F	1	5.95	P4D	A	0367Red	769005B	na	capture
9/01/2015	539646	6794219	M	na	8.2	P4D	A	0388Red	7710876	na	capture
9/01/2015	539581	6794223	M	na	5.8	P4E	A	0387Red	771066C	na	capture
11/01/2015	544394	6796993	F	2	6.3	P4E	A	0369Red	77104BC	na	capture
27/01/2015	538387	6796522	F	2	7.3	P4E	A	na	768FECE	TVWC 026	capture
6/02/2015	538439	6796479	M	na	7.25	P4D	A	0362Red	7710BEB	na	capture
17/02/2015	541183	6793594	M	na	5.95	P4D	A	0361Red	76937AE	na	capture
17/02/2015	542553	6792693	M	na	7.2	P4E	A	0364Red	768FDDF	na	capture
18/02/2015	541199	6793976	F	3	5.05	P4E	A	0363Red	7710665	na	capture
18/02/2015	542577	6792692	F	3	5.15	P4D	A	0366Red	770E9C9	na	capture

DoC	Easting	Northing	Sex	FRS	Weight (kg)	TWS	COH	Tag	MCHIP	Other ID	Detail
27/02/2015	538421	6796349	F	1	4.9	P4D	A	na	na	FoK8039	capture
26/02/2015	541933	6793709	M	na	7.85	P4FM1H	A	0379Red	770C8C2	na	capture
26/02/2015	541092	6793851	F	1	na	P4B	SA	na	na	260215_01	body in tree
27/02/2015	541212	6794012	M	na	6.1	P4D	A	0365Red	76905FE	na	capture
4/03/2015	538391	6796435	F	1	5.12	P4D	A	0372Red	76919B5	na	capture
5/03/2015	538422	6796261	M	na	8.17	P4E	A	0380Red	770FD1A	na	capture
5/03/2015	539682	6793884	F	3	6.1	P4E	A	0371Red	770CB5E	na	capture
13/03/2015	542802	6798218	F	1	5.71	P4FM1G	A	0345Red	770C794	na	capture
25/03/2015	544768	6797159	F	3	5.25	P4C	A	0346Red	770C766	na	capture
28/03/2015	540625	6793834	M	na	1.8	P4B	SA	0347Red	770C727	na	capture
8/04/2015	542330	6799857	F	1	5.25	P4FM1H	A	0348Red	na	na	capture
13/04/2015	539626	6794227	M	na	na	P4D	A	na	na	130415_01	road- kill
15/04/2015	546278	6799664	F	1	4.6	P4D	A	na	na	140415_01	road-kill
16/04/2015	543774	6806077	M	na	7	P4D	A	0349Red	na	na	capture
17/04/2015	541421	6798534	F	1	5.45	P4FM1G	A	0350Red	na	na	capture
17/04/2015	543577	6803659	F	1	5.92	P4FM1IM2H	A	0351Red	770CA87	na	capture
23/04/2015	542778	6798561	F	2	4.85	P4C	A	0352Red	7691358	na	capture
30/04/2015	547016	6806988	F	1	na	-	A	na	na	na	road-kill
5/05/2015	542496	6799656	F	3	6.25	P4E	A	0353Red	na	na	capture
8/05/2015	542983	6792452	F	3	6.05	P4D	A	0389Red	na	na	capture
9/05/2015	541959	6804916	F	3	6.06	P4C	A	0390Red	na	150508	road-kill
14/05/2015	539567	6794192	F	1	5.1	P4D	A	0391Red	na	na	capture
14/05/2015	542668	6801601	F	1	2.77	P4B	SA	0392Red	7690717	na	capture
21/05/2015	540608	6806450	F	1	4.8	P4D	A	0393Red	na	CSWH 40192	capture
22/05/2015	540641	6806535	M	na	3.32	P4B	SA	0394Red	770E7E2	na	capture

DoC	Easting	Northing	Sex	FRS	Weight (kg)	TWS	COH	Tag	MCHIP	Other ID	Detail
23/05/2015	540724	6806511	F	3	7.32	P4E	A	0395Red	na	na	capture
23/05/2015	540657	6806518	F	1	3.1	P4B	SA	0396Red	768FAF3	na	capture
27/05/2015	543426	6805606	F	1	6.45	P4C	A	0397Red	770D286	na	capture
11/06/2015	541771	6799825	M	na	7.69	P4FM1H	A	0398Red	na	na	capture
29/06/2015	539580	6794311	M	na	6.65	P4D	A	na	na	01_28062015	killed by dog/fox
7/07/2015	545796	6798190	F	1	4.2	P4E	A	na	na	na	Domestic dog attack

Appendix 2 Transect data

Site No.	Ha	n	K	Easting	Northing
ES Feb10_5	1	1	0	546264.213	6800030.86
ES Feb10_6	1	1	0	541764.113	6798521.401
ES Feb10_7	1	1	1	542764.11	6798521.40
ES Feb10_8	1	1	0	542764.11	6798021.40
ES Feb10_9	1	1	0	541774.82	6797522.11
ES Feb10_10	0.36	1	0	542764.11	6797521.40
ES Feb10_12	1	1	0	542764.11	6794521.40
ES Feb10_13	1	1	0	542764.11	6794021.40
ES Feb10_14	1	1	0	542764.11	6793521.40
ES Feb10_15	1	1	0	542264.11	6793021.40
ES Feb10_16	1	1	0	542764.11	6793021.40
ES Feb10_18	1	1	0	542765.27	6792520.78
ES Apr10_5	1	1	0	542751.16	6800010.67
ES Apr10_9	1	1	0	543751.16	6799510.67
ES Apr10_10	1	1	0	544751.16	6799510.67
ES Apr10_12	1	1	0	542751.16	6799010.67
ES Apr10_18	1	1	0	541251.16	6795010.67
ES Apr10_19	1	1	1	541251.16	6794010.67
0.5K 008	1	1	0	540251.16	6797010.67
0.5K 014	1	1	1	539251.16	6796010.67
0.5K 055	1	1	0	542251.16	6794010.67
0.5K 057	1	1	0	543251.16	6794010.67
0.5K 064	1	1	0	541751.16	6794510.67
0.5K 085	1	1	0	541751.16	6795010.67
0.5K 086	1	1	0	542251.16	6795010.67
0.5K 087	1	1	0	541751.16	6796010.67
0.5K 088	1	1	0	541751.16	6796510.67
0.5K 089	1	1	0	542251.16	6797010.67
1K 002	1	1	0	541251.16	6793510.67
1K 003	1	1	0	542251.16	6793510.67
1K 004	1	1	0	543251.16	6793510.67
1K 007	1	1	0	542251.16	6794510.67
1K 011	1	1	0	542251.16	6795510.67
1K 013	1	1	0	538251.16	6796510.67
1K 014	1	1	0	539251.16	6796510.67
1K 016	1	1	0	542251.16	6796510.67
1K 017	1	1	0	544251.16	6796510.67

Site No.	Ha	n	K	Easting	Northing
1K 020	1	1	0	538251.16	6797510.67
1K 023	0.98	1	0	542251.16	6797510.67
1K 024	1	1	0	543251.16	6797510.67
1K 025	1	1	0	544251.16	6797510.67
1K 027	1	1	0	540251.16	6798510.67
1K 030	1	1	0	540251.16	6799510.67
1K 031	1	1	0	546251.16	6799510.67
1K 033	1	1	0	542251.16	6800510.67
1K 036(b)	1	1	0	546313.00	6800372.00

Appendix 3 Field sites – Wardell koala meta-population survey

Site	Type	Easting	Northing	p	n	Activity %
1	Primary	542774	6792530	2	30	6.67
2	Primary	542541	6792763	10	25	40.00
3	Primary	542990	6792758	0	27	0.00
4	Primary	542749	6793027	0	25	0.00
5	Primary	542483	6793245	0	25	0.00
6	Primary	542996	6793284	1	26	3.85
7	Primary	542784	6793538	5	30	16.67
8	Primary	543244	6793524	0	26	0.00
9	Primary	542479	6793726	2	30	6.67
10	Primary	542997	6793821	1	27	3.70
11	Primary	542663	6793899	0	25	0.00
12	Primary	543217	6793968	3	30	10.00
13	Primary	542489	6794316	0	29	0.00
14	Primary	543024	6794270	2	30	6.67
15	Primary	543501	6794274	0	26	0.00
16	Primary	542205	6794527	1	30	3.33
19	Primary	542550	6794778	0	25	0.00
20	Primary	543494	6794777	0	26	0.00
21	Primary	542249	67955083	4	30	13.33
22	Primary	543723	6795017	0	26	0.00
23	Primary	542487	6795273	0	25	0.00
24	Primary	542230	6795514	2	30	6.67
25	Primary	543762	6795512	11	22	50.00
26	Primary	542017	6795811	0	25	0.00
28	Primary	543785	6795995	0	26	0.00
29	Primary	541988	6796286	6	30	20.00
32	Primary	541967	6796767	7	30	23.33
36	Primary	541917	6797287	10	27	37.04
39	Primary	544074	6797280	0	26	0.00
40	Primary	542259	6797542	1	30	3.33
44	Primary	544237	6797602	3	30	10.00
45	Primary	544760	6797511	3	30	10.00
46	Primary	542471	6797792	0	25	0.00
48	Primary	543455	6797739	4	30	13.33
49	Primary	542724	6798051	7	30	23.33
50	Primary	543742	6798050	4	30	13.33
51	Primary	544148	6797982	0	26	0.00
52	Primary	544702	6797956	1	30	3.33
53	Primary	545247	6798069	2	30	6.67
54	Primary	542516	6798234	2	30	6.67

Site	Type	Easting	Northing	p	n	Activity %
55	Primary	542927	6798226	1	28	3.57
57	Primary	542755	6798542	12	18	66.67
58	Primary	542539	6798782	13	13	100.00
62	Primary	543520	6799356	0	26	0.00
63	Primary	544462	6799231	0	26	0.00
64	Primary	544899	6799313	0	26	0.00
65	Primary	545596	6799244	0	26	0.00
66	Primary	543733	6799513	0	26	0.00
68	Primary	546019	6799766	0	26	0.00
69	Primary	546208	6800005	2	30	6.67
83	Primary	542957	6792354	13	27	48.15
87	Primary	543997	6796766	0	24	0.00
102	Primary	544511	6797240	0	29	0.00
18	Supplementary	543491	6794529	0	26	0.00
31	Supplementary	544270	6796520	1	26	3.85
33	Supplementary	544389	6796647	2	30	6.67
70	Supplementary	546303	6799815	10	23	43.48
71	Supplementary	542498	6795551	11	19	57.89
72	Supplementary	542344	6795454	3	30	10.00
73	Supplementary	542654	6792885	7	30	23.33
74	Supplementary	542615	6792652	7	30	23.33
75	Supplementary	543898	6795636	12	30	40.00
76	Supplementary	543625	6795644	0	27	0.00
77	Supplementary	543569	6795435	11	30	36.67
84	Supplementary	542863	6792392	2	30	6.67
85	Supplementary	544487	6797030	13	25	52.00
86	Supplementary	544336	6794727	2	29	6.90
88	Supplementary	544279	6796283	17	27	62.96
89	Supplementary	542759	6792762	3	29	10.34
90	Supplementary	542986	6792500	12	29	41.38
92	Supplementary	543638	6795116	0	27	0.00
94	Supplementary	543497	6795502	4	30	13.33
97	Supplementary	543906	6795865	0	26	0.00
99	Supplementary	542854	6792626	1	27	3.70
100	Supplementary	542250	6796457	9	30	30.0
101	Supplementary	542202	6796045	9	30	30.0

Revision History

Revision No.	Revision date	Details	Prepared by	Approved by
00	10/07/2015	Koala Genetics and Population Demographic Surveys, Woolgoolga to Ballina Pacific Highway Upgrade: Section 10 (Wardell to Coolgardie) - Draft	Steve Phillips, Managing Director/Principal Ecologist (Biolink); Grant Brearley, Senior Ecologist (Ecosure); and John Callaghan, Director/Conservation Biologist (Biolink)	Steve Phillips (Biolink)
01	24/08/2015	Incorporates comments on the Draft from Roads and Maritime Services, Associate Professor Jonathon Rhodes (University of Queensland) and Dr Rod Kavanagh (Niche Consulting)	Steve Phillips, Managing Director/Principal Ecologist (Biolink); Grant Brearley, Senior Ecologist (Ecosure); and John Callaghan, Director/Conservation Biologist (Biolink)	Steve Phillips (Biolink)
02	02/10/2015	Incorporates further comments on the Revised Draft from Roads and Maritime Services, Associate Professor Jonathon Rhodes (University of Queensland) and Dr Rod Kavanagh (Niche Consulting)	Steve Phillips, Managing Director/Principal Ecologist (Biolink); Grant Brearley, Senior Ecologist (Ecosure); and John Callaghan, Director/Conservation Biologist (Biolink)	Steve Phillips (Biolink)
Final	03/12/2015	Incorporates final comments on the Revised Draft from Roads and Maritime Services and Associate Professor Jonathon Rhodes (University of Queensland)	Steve Phillips, Managing Director/Principal Ecologist (Biolink); Grant Brearley, Senior Ecologist (Ecosure); and John Callaghan, Director/Conservation Biologist (Biolink)	Steve Phillips (Biolink)

Distribution List

Copy #	Date	Type	Issued to	Name
1	03/12/2015	Electronic	Roads and Maritime Services	Simon Wilson
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3	03/12/2015	Electronic	Biolink	Steve Phillips

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Report compiled by Biolink Ecological Consultants and Ecosure Pty Ltd

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Appendix 2 – Southern Cross University genetics report

Southern Cross University

Genetic profiling of koalas:
Woolgoolga to Ballina Pacific Highway Upgrade
(Section 10–Wardell to Coolgardie)

Southern Cross University

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August 2015

CITATION: Norman, J.A., Blackmore, C., Goldingay, R. and Christidis, L. 2015. *Genetic profiling of koalas: Woolgoolga to Ballina Pacific Highway Upgrade (Section 10 – Wardell to Coolgardie)*. Final report to NSW Roads and Maritime Services. Southern Cross University.

Executive summary

The southern sector of the Ballina Local Government Area (LGA) has been identified as supporting an important population of around 200 koalas: the Wardell Koala Metapopulation (Wardell KMP) for *Environment Protection and Biodiversity Conservation Act 1999* purposes. Resident koala populations occur near Coolgardie, Wardell, Bagotville and Meerschaum Vale in two large tracts of remnant schlerophyll woodland on the south-eastern boundary of the former Big Scrub habitat. The Wardell KMP may be a significant source population for koala dispersal into this area which has been colonised following extensive logging of the native rainforest vegetation and conversion to agricultural land.

Section 10 of the conditionally-approved Pacific Highway upgrade will traverse the Wardell KMP and bisect the Bagotville area previously identified as containing important remnant habitat for koala dispersal. Consequently, there is potential for the realignment to disrupt dispersal within the Wardell KMP and impact regional koala population dynamics.

Genetic profiling of the Wardell KMP was undertaken by Southern Cross University (SCU) as part of the project Genetics and Population Demographics of Koalas Inhabiting Coastal Lowlands in the Ballina Local Government Area – Stage 10 Pacific Highway Upgrade. Genetic profiling was conducted using microsatellites in order to describe patterns of population structure and dispersal within the Wardell KMP and determine its regional significance as a source population.

The Wardell KMP was found to be spatially structured. Dispersal, estimated from genetic neighbourhood size and the distribution of first-order relatives (FOR), largely conformed to an isolation-by-distance model. However, we found evidence of more substantial genetic differentiation between the Northern and Southern subpopulations of the Wardell KMP with higher genetic variation and lower mean relatedness (for both males and females) in the north. This was unexpected given the geographic proximity of these subpopulations and evidence of ongoing dispersal between them. The most likely explanation is that the Northern subpopulation receives immigrants from koala populations in surrounding areas. Immigration into the Southern subpopulation appears to be limited in comparison.

These findings emphasise the potential significance of the Southern subpopulation in the vicinity of Bagotville as the main source population for habitats to the west in the area of the former Big Scrub rainforest. Although multiple source populations are indicated, spatial patterns of dispersal suggest substantially higher levels of connectivity are maintained with the Southern Wardell subpopulation. Dispersal is most likely asymmetric, occurring predominantly from the Southern Wardell subpopulation into the adjacent areas of the former Big Scrub habitat.

The Southern subpopulation occurs in an area of remnant schlerophyll woodland that will be bisected by the conditionally-approved Section 10 Pacific Highway upgrade. Genetic profiling confirmed dispersal of koalas across the proposed route. The Pacific Highway upgrade will also traverse areas occupied by the Northern subpopulation, between Wardell and Meerschaum Vale, with potential dispersal of koalas across the intervening valley floor indicated. These findings highlight the need for road-crossing structures to enable koala dispersal.

The findings presented in this report are intended to inform a concurrent Population Viability Assessment (PVA) undertaken as part of the project Genetics and Population Demographics of Koalas Inhabiting Coastal Lowlands in the Ballina Local Government Area – Stage 10 Pacific Highway Upgrade.

Acknowledgements

We wish to thank Biolink Ecological Consultants, Ecosure Pty Ltd and Roads and Maritime Services for the opportunity to contribute to the project Genetics and Population Demographics of Koalas Inhabiting Coastal Lowlands in the Ballina Local Government Area – Stage 10 Pacific Highway Upgrade. We gratefully acknowledge Biolink Ecological Consultants for the collection of koala tissue samples obtained during their extensive survey work. Also, Friends of the Koala Inc. who made additional samples available to Southern Cross University which we have included in the study as unpublished data. We thank Steve Phillips (Biolink Ecological Consultants) for helpful discussions and background information, and staff at the National Marine Science Centre, Southern Cross University, for their invaluable logistical and administrative support. The project was funded by Roads and Maritime Services.

Acronyms and abbreviations

CI	confidence interval
DNA	deoxyribonucleic Acid
FoK	Friends of the Koala Inc.
FOR	first-order relatives
GPA	generational persistence assessment
ha	hectare
IP	Important Population
km	kilometre(s)
KMP	Koala Metapopulation
LGA	Local Government Area
NSW	New South Wales
PVA	population viability assessment
SCU	Southern Cross University

Glossary of genetic terms

Coefficient of relatedness: a measure of the percentage of genes shared by two individuals. For first-order relatives (e.g. a mother and her offspring) this coefficient is expected to be 0.5 as the offspring obtains half their genes from the mother and half from the father. In natural populations the estimated value for first-order relatives can vary depending on the extent of inbreeding and the variability of the genetic markers examined.

F-statistics: describe the extent of genetic differentiation between populations caused by a reduction in observed heterozygosity. Heterozygosity is measured at three hierarchical levels (within individuals in each subpopulation, within subpopulations and across the total population). When a population is structured heterozygosity within subpopulations is lower than estimated for the total population. Values can range from 0.0 (no differentiation) to 1.0 (complete differentiation). In the latter case subpopulations would not share any genetic variants at the surveyed microsatellites.

Hardy-Weinberg equilibrium: in a randomly mating population genetic variants are expected to be maintained in a particular ratio of homozygotes and heterozygotes. Departures from this ratio may indicate that the genetic markers being examined are under selection which can limit their usefulness in population studies.

Stepwise mutation model: assumes that variation in microsatellites arise from a step-wise increase or decrease in repeat length. In this model repeats of similar length are considered closely related. The alternative model assumes that changes in repeat length are unconstrained such that repeats of similar size may be unrelated.

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Introduction

1.1 Project background

This report presents the results of the genetic profiling component of a project which examined the distribution, density, demographics and genetics of a koala population known to be present within approximately 8,250 ha of coastal lowlands in the Ballina Local Government Area (LGA). The project was commissioned by the New South Wales Roads and Maritime Services as part of koala preconstruction surveys associated with the conditionally-approved Woolgoolga to Ballina Pacific Highway Section 10 upgrade. Biolink Ecological Consultants and Ecosure Pty Ltd completed the koala demographic study (Phillips et al. 2015) which provided the genetic material that forms the basis of the present study. The genetic profiling project has been undertaken by Southern Cross University (SCU).

Section 10 will be traversing an area of koala habitat in the southern sector of the Ballina LGA identified as supporting an important koala population; the Wardell Koala Metapopulation (Wardell KMP) for *Environment Protection and Biodiversity Conservation Act 1999* purposes (Phillips & Chang 2013; Ecosure 2014). The LGA supports multiple landuse types including native forests, plantations, agricultural landscapes and residential areas. The northern extent occurs in highly modified habitat that was formerly part of the Big Scrub subtropical rainforest whereas southern areas are dominated by heathland and areas schlerophyll woodland and forest. Preferred koala habitat across the LGA is largely fragmented and was estimated to comprise 1,500 – 2,000 ha (Phillips & Chang 2013) of the total area.

Population distribution modelling based on Generational Persistence Assessments (GPA) and koala activity patterns confirm the long-standing presence of resident koala populations associated with two large tracts of woodland in the vicinity of Coolgardie, Wardell, Bagotville and Meerschaum Vale (Phillips & Chang 2013). The Bagotville area bisected by the conditionally-approved Section 10 Pacific Highway upgrade has been identified as containing important remnant habitat for koala dispersal (Ecosure 2014, Phillips & Chang 2013). Resident koala populations are also present in the vicinity of Uralba and Lynwood in highly modified agricultural landscapes to the north and north-west of these forested areas. For the purposes of this study and an associated PVA the Wardell KMP encompasses these resident populations along with habitat in the vicinity of Dalwood, a north-western extension of the original Important Population (IP) boundary defined by Phillips & Chang (2013).

Koala density data reported by Phillips & Chang (2013) enable a population estimate of approximately 200 animals for the Wardell KMP. Based on the long-term persistence of this metapopulation, and increases in the extent of area occupied by koalas in the adjacent Lismore and Byron LGA's, it has been suggested that the Wardell KMP may be an important source population for these areas (Phillips & Chang 2013). The Wardell KMP is already subject to ongoing threats from road mortality, habitat loss, dog attacks and disease. There is potential for Section 10 to have significant additional impacts (Phillips & Chang 2013) to the detriment of regional koala population dynamics.

1.2 Scope of work

This project uses genetic profiling to describe patterns of population structure and gene dispersal within the Wardell KMP and determine its regional importance as a source population for surrounding areas. The results are intended to inform a concurrent PVA and provide baseline information on levels of genetic variation for future monitoring. Two main issues were considered:

1. Is the Wardell KMP spatially structured?

For species which have limited dispersal capabilities, populations are expected to be spatially structured. Where dispersal into new territories is spatially uniform this leads to a pattern of isolation-by-distance in which related individuals occur in close proximity and the degree of relatedness declines with distance. Physical barriers to dispersal, or the presence of dispersal corridors, can lead to departures from a strict isolation-by-distance model of population structure over relatively small spatial scales. Dispersal away from the natal site is considered to be somewhat limited in koalas (~3.5 km) and sex biased, with a higher proportion of males than females migrating to new territories (Dique et al 2003). For the purposes of this study we used estimates of genetic relatedness to determine if limited dispersal leads to patterns of population substructure within the Wardell KMP. The results will assist in identifying areas of high natural connectivity that may be impacted by the conditionally-approved Section 10 upgrade. The presence of population substructure and inferred dispersal patterns should also be accounted for in the associated PVA.

2. Is the Wardell KMP an important source population for surrounding areas?

The Wardell KMP is located on the south-eastern boundary of the former Big Scrub, an area that previously supported large tracts of subtropical rainforest. The Big Scrub was extensively logged and converted to agricultural use by the early 20th century (Lott & Duggin 1993). Since then much of the area has been colonised by koalas, presumably a result of the planting of windbreaks with koala food tree species such as Tallowwood (*Eucalyptus microcorys*) that may have functioned to facilitate dispersal from surrounding areas (Phillips & Chang 2013). For the purposes of this study we used genetic profiling to test whether koala populations in the adjoining habitat to the west are related to those in the Wardell KMP, or to other regional koala populations (SCU unpublished data). If the Wardell KMP is a significant source population for these surrounding areas the conditionally-approved Section 10 upgrade could potentially impact regional koala population dynamics.

Methods

2.1 Tissue samples

Tissue samples for DNA analysis were collected from ear punches of 40 koalas captured (or found deceased) during Roads and Maritime preconstruction surveys of the Wardell KMP. An additional 11 samples were made available by SCU from samples collected on their behalf by the Friends of the Koala Inc. (FoK). Post-mortem ear biopsies were obtained from these animals which had died as a result of disease, injury or unknown causes.

Six koala scats were also collected but time constraints precluded their inclusion in the present study. Extraction of DNA from scats is more difficult and requires modifications to standard extraction protocols to concentrate the trace amounts of DNA that may be present and eliminate inhibitory substances. Furthermore, the quality of DNA obtained from scats is very dependent on the length and type of environmental exposure prior to collection and it is recommended to perform genetic profiling on freshly collected scats (Wedrowicz et al. 2013). Genetic profiles obtained from scat DNA are also subject to a high error rate. Although reliable profiles can be obtained by running each sample in triplicate (Wedrowicz et al. 2013) this imposes considerable additional time and costs that were not available.

To investigate regional koala population dynamics genetic profiles of an additional 88 koalas were made available (SCU, unpublished data). This included genetic profiles of 42 koalas from habitat areas to the west of Lismore and the Wardell KMP. Genetic profiles were also provided for koalas inhabiting outlying areas to the northeast (30) and southwest (16) of Lismore.

2.2 Genetic profiling

Genetic profiling was performed at the Australian Genome Research Facility using DNA extracted at SCU from koala tissue samples using the QIAGEN DNeasy Blood and Tissue Kit. To obtain unique genetic profiles for each sample sections of DNA that contained *short tandem repeats* (e.g. CACACACA) were examined. These regions are known as *microsatellites* and are characterised by variation in repeat length. Each animal carries two copies of a microsatellite, one inherited from their sire (father) and one from their dam (mother). The copies may be identical in repeat length (homozygous) or may be a different length (heterozygous). Microsatellites that are heterozygous within individuals are the most useful for genetic profiling.

For this study we utilised 14 published koala microsatellites with average observed heterozygosities in the range 0.255 to 0.894 (mean 0.631) for the Wardell KMP. Together, these fourteen microsatellites have an exclusion probability when one parent is known of 99.97546, and an exclusion probability when both parents are unknown of 98.95176. The single known example of a parent-offspring pairing returned a relatedness coefficient (R) of 0.405, close to the expected mean of 0.5 for first order relatives (FOR; parent-offspring, full-siblings). All microsatellites except one were found to be in Hardy-Weinberg equilibrium with post-hoc testing showing this departure was due to spatial structure within the sample. We determined that this set of microsatellites are able to detect the presence of genetic differentiation amongst populations with a power of 0.975 or higher after 10 generations and assuming an effective population size of 50-200 (approximately the number of reproductive adults per population which is smaller than the census population size). Thus, the 14 microsatellites were confirmed as suitable for analysis of local and regional population structure, estimation of genetic diversity, and analysis of the relatedness of individuals within and between populations.

Analyses of the Wardell KMP that follow are based on the genetic profiles obtained for 47 of the 51 koalas sampled; three FoK samples being excluded from the analysis as genetic profiling showed that they were among the koalas that had been sampled earlier during the Roads and Maritime surveys. The single joey of a deceased female was also excluded from the population analysis to avoid bias.

2.3 Data analysis

2.3.1 Spatial structure within the Wardell KMP

To determine if there was evidence of spatial structure within the Wardell KMP we first employed the distance measure *Aij* (Rousett 2000) to estimate the size (geographic extent in km^2) of genetic neighbourhoods in the study area. This approach defines the geographic range over which gene dispersal occurs and identifies the likely occurrence of clusters of closely related individuals. Geospatial data was provided as decimal degrees and the analysis run under different assumptions of koala population density to simulate a population ranging in size from

93-930 individuals. This approach broadly encompasses the current estimate of approximately 200 koalas in the Wardell KMP.

Population substructure was subsequently assessed from the spatial distribution of FOR identified using the relatedness coefficient (R) of Wang (2002) which performs well in structured populations. The 51 koala DNA samples were assigned to eight sample groups defined on the basis of habitat type, focal areas of koala activity (Phillips & Chang 2013) and patterns of intergenerational persistence (Phillips & Chang 2013) (Table 1, figure 1). Partitioning of the samples in this way allows the following questions to be addressed:

1. Do focal areas of koala activity support discrete subpopulations?
2. Are samples to the east and west of the Section 10 upgrade related and connected by high levels of contemporary dispersal?
3. Do alternative patterns of spatial structure exist within the Wardell KMP?

Table 1. Assignment of Wardell KMP DNA samples to groups based on geographic proximity, habitat type and ecological attributes. * data sourced from Phillips & Chang (2013). # habitat occupied by koalas in sample group 2b is bisected by the conditionally-approved Section 10 upgrade.

Sample grouping	Location	Sample size	Habitat Type	Focal Area*	GPA*
1a	Lynwood	6	Agriculture	A	High
1b	Dalwood	7	Agriculture	-	Low
2a	Wardell	6	Forest	B	High
2b#	East Bagotville	10	Forest	B	high
3a	Coolgardie	4	Forest	C	High
3b	Meerschaum Vale	4	Agriculture/Forest	D	High
4a	West Bagotville 1	3	Forest fragment	D	Low
4b	West Bagotville 2	7	Forest fragment	D	Low

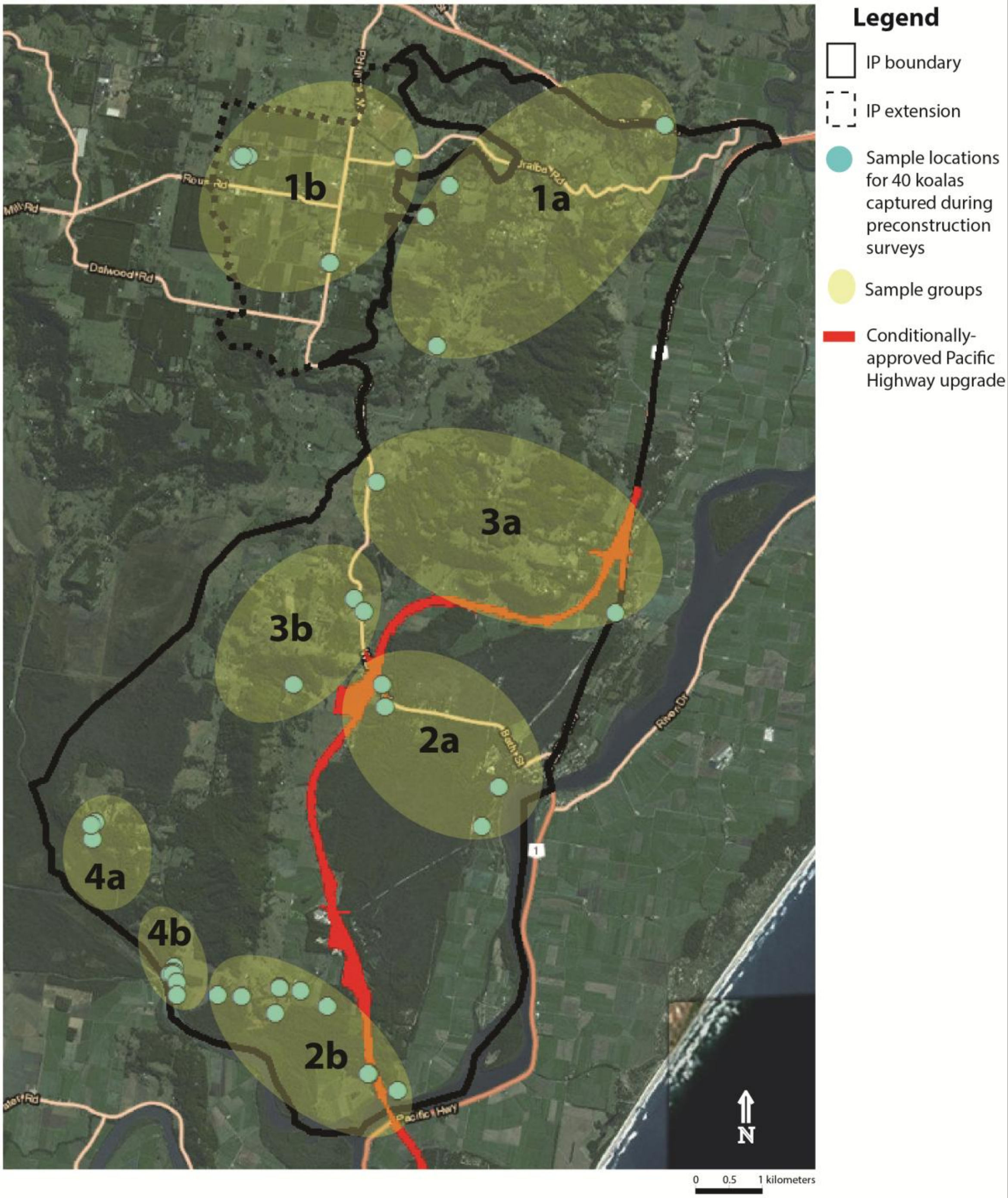

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Figure 1. Wardell koala metapopulation boundary, sample locations and sample groups used to infer dispersal patterns

Pairwise relatedness (R) was estimated for all individuals within and amongst the eight groups. Potential FOR were identified using the theoretical mean R of 0.5 ± 1 standard deviation (range 0.378 – 0.622) following Blouin et al. (1996). Under limited dispersal FOR are expected to occur in close proximity, within groups or between adjacent groups as defined in the present analysis. Distant groups are not expected to share FOR unless occasional long-distance dispersal occurs. The spatial distribution of FOR was mapped to show likely patterns of contemporary dispersal within the Wardell KMP.

From the inferred patterns of dispersal sample groups were aggregated into logical clusters and the difference in mean R for each pair of clusters determined. For the purpose of this study clusters were considered to be different if this value lay outside the 95% confidence interval (CI) for the cumulative frequency of R values obtained by bootstrap resampling of the data. A significant difference indicates underlying differences in the demographic structure of the sampled populations which affect levels of koala relatedness (e.g. the extent of inbreeding and levels of immigration and/or emigration). We extended this approach to test for differences in mean R between males and females across the Wardell KMP. Sex-biased dispersal, in which males disperse further than females, has been reported for koalas (Dique et al 2003). This should lead to female koalas being more closely related than males within a population or subpopulation.

2.3.2 Regional population structure and the significance of the Wardell KMP as a source population

We used pairwise estimates of R (as described above) to test for evidence of dispersal between the Wardell KMP and populations present in areas of the former Big Scrub habitat to the west. Samples from the Big Scrub were provided by SCU and grouped into a South Lismore population (South Lismore-Alstonville) and a Western population (Wyrallah-East Coraki) (Figure 2).

The extent of genetic differentiation amongst regional populations and subpopulations was also assessed using pairwise F-statistics; *Fst* (Weir & Cockerham 1984) and *Rst* (Michalakis & Excoffier 1996). F-statistics quantify the extent to which population subdivision leads to a reduction in observed heterozygosity. *Rst* is a more suitable estimator for microsatellites as it assumes a stepwise mutation model. However, previous koala genetic studies have employed *Fst* to estimate levels of population differentiation. We have included values for both estimators to allow comparison with published estimates of genetic differentiation amongst regional koala populations in New South Wales and South-east Queensland. Outlying populations to the north (North Lismore) and west of Lismore (Far-western) (SCU, unpublished data; Figure 2) were also included in the analysis. The North Lismore population occurs in an area of the Big Scrub rainforest colonised by southward dispersal of koalas from South-east Queensland (Lee et al. 2013).

2.3.3 Genetic variation

Summary statistics that describe levels of genetic variation were calculated for the Wardell KMP: observed (Ho) and expected (He) heterozygosity, an unbiased estimator of the effective number of alleles (AE), and an inbreeding coefficient (Fi).

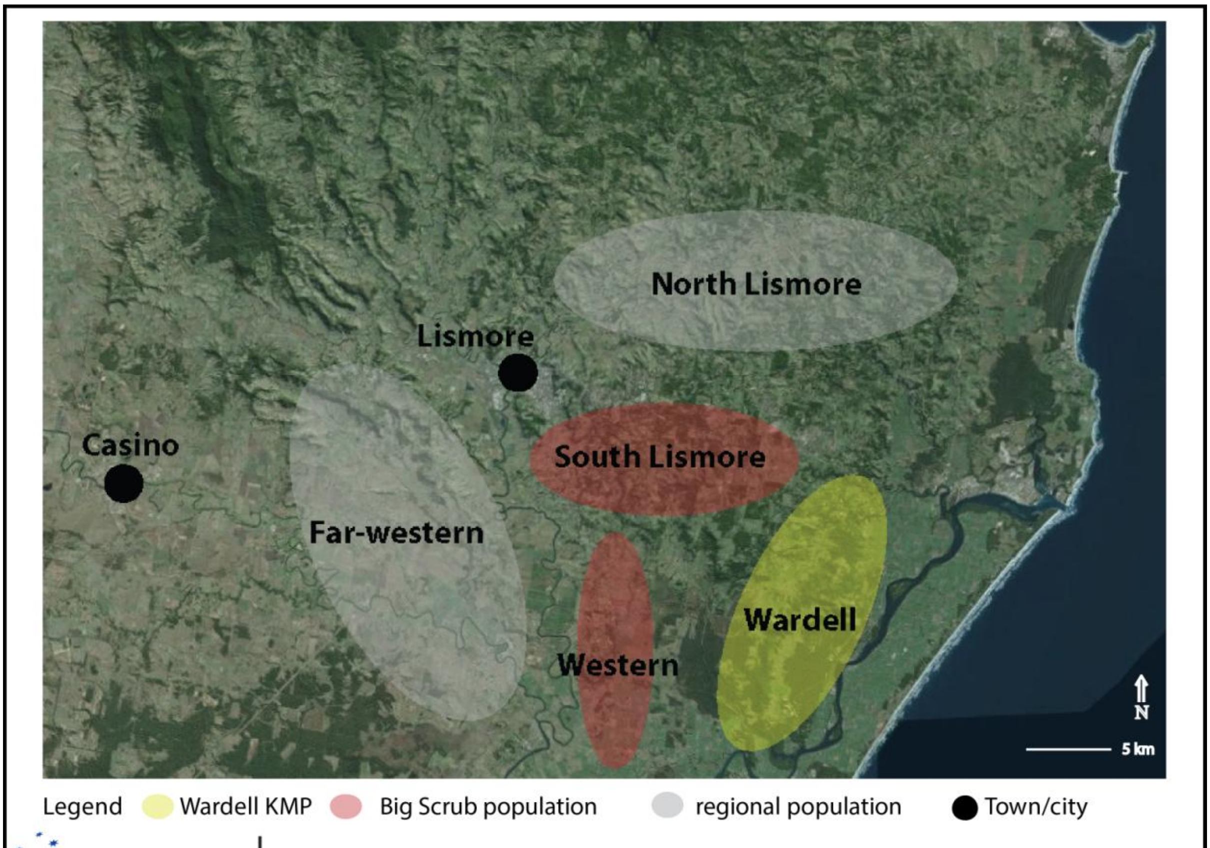

Figure 2. Population designations for analysis of regional koala population dynamics

Results

3.1 Spatial structure within the Wardell KMP

Genetic neighbourhood size in the Wardell KMP was estimated to be 21-30 Km^2. This confirms a pattern of limited dispersal across the study area and the likely presence of multiple subpopulations. The estimated genetic neighbourhood size suggests that koalas within the Wardell KMP typically disperse distances of 4.5-5.5 km from the natal site. This is broadly consistent with published dispersal estimates of 3.5 km for dispersing young in a peri-urban landscape in south-east Queensland (Dique et al 2003).

Analysis of the spatial distribution of FOR amongst the eight sample groups approximates an isolation-by-distance model with a high proportion of FOR occurring within (37.8%) or between adjacent groups (48.9%) (Figure 3a). This included a small number of comparisons in which R values exceeded the upper threshold of 0.622. This could reflect our application of a conservative threshold for FOR or indicate occasional mating between related animals. In the Bagotville area a large number of FOR were shared amongst the three sample groups (2b, 4a and 4b). We also detected evidence of dispersal across the area where the conditionally-approved Section 10 upgrade bisects this large tract of remnant habitat (Figure 3a inset). Potential dispersal across the valley floor was detected in the vicinity of Wardell and Meerschaum Vale (Figure 3a), an area also bisected by the conditionally-approved Section 10 upgrade.

Based on the observed patterns three potential subpopulation clusters were identified; a northern cluster comprising koalas sampled from Lynwood (1a), Coolgardie (3a) and Dalwood (1b), a central cluster comprising koalas from Wardell (2a) and Meerschaum Vale (3b), and a southern cluster comprising koalas from the Bagotville area (2b, 4a and 4b) (Figure 3a). FOR were also detected between these three clusters (13.3%) (Figure 3b) indicating the occurrence of infrequent long-distance dispersal events across the study area of up to 15 kilometres.

The significance of the observed spatial patterns were assessed using the 95% CI of the cumulative mean frequency obtained from pairwise comparisons of R for the three clusters. Only two of the three tests were significant; the mean difference in R for the northern and central clusters falling within (but close to) the 95% CI (Table

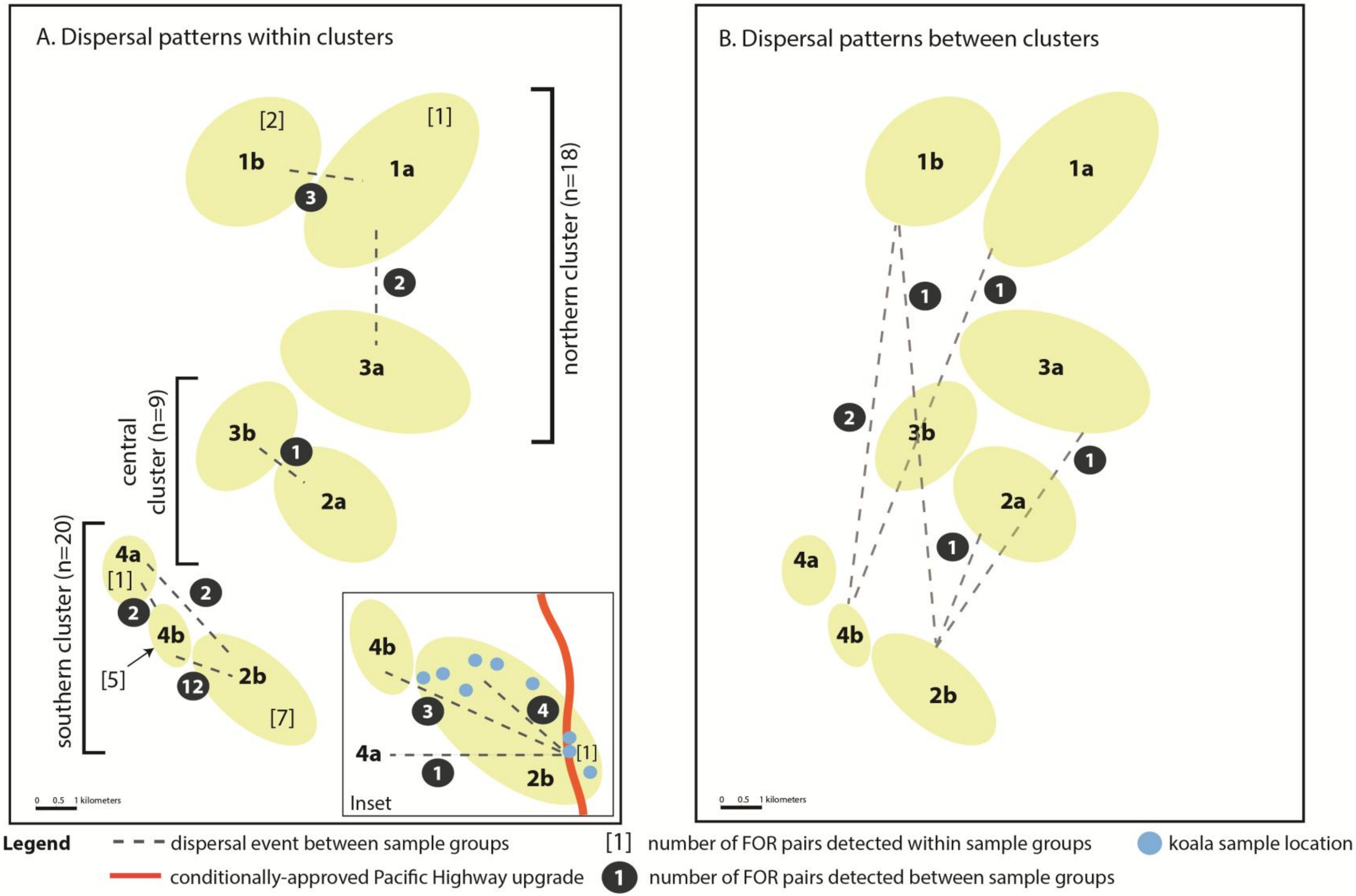

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Figure 3. Patterns of dispersal within the Wardell KMP and across the Section 10 Pacific Highway upgrade (inset)

2). Based on these results a hierarchical population structure is indicated with the Wardell KMP comprising two main subpopulations:

1. a Northern subpopulation provisionally containing the northern and central clusters;
2. a Southern subpopulation located in the Bagotville area.

Consistent with this arrangement we also found differences in R when comparing the demographic profiles of the two subpopulations. Both males and females in the Southern subpopulation are, on average, more closely related than those in the Northern subpopulation (Table 3). Within the Southern subpopulation the mean R for females (~0.20) was twice that of males (~0.09) a pattern consistent with more limited dispersal of females away from the natal site.

Table 2. Mean difference in relatedness (R) between sample clusters in the Wardell KMP. Differences are significant if the mean falls outside the 95% CI (2.5-97.5% quantiles).

Cluster 1	Cluster 2	Mean difference	2.5% quantile	97.5% quantile
northern	central	0.0676	-0.0618	0.0685
central	southern	0.1939	-0.0713	0.0653
northern	southern	-0.1121	-0.0607	0.0639

Table 3. Mean difference in relatedness (R) between males and females in the Wardell KMP. Differences are significant if the mean falls outside the 95% CI (2.5-97,5% quantiles)

Comparison: (A cf. B)	Mean R A	Mean R B	Δ Mean R	95% CI
Across Wardell KMP: males cf. females	-0.0068	-0.0234	0.0166	-0.0422 – 0.0399
Males: Northern cf. Southern	-0.0974	0.0887	0.1862	-0.1719 – 0.1835
Females: Northern cf. Southern	-0.0289	0.1973	0.2262	-0.0809 – 0.0830

The proposed subdivision of the Wardell KMP into a Northern and Southern subpopulation does not support models of population structure based solely on areas of focal activity, habitat type or landuse. The Northern subpopulation occupies both agricultural landscapes and contiguous native forest, contains three focal areas of activity, and encompasses areas of high and low generational persistence (Table 2). The Southern subpopulation is largely confined to fragmented and contiguous forest habitats and also contains multiple focal areas of activity and areas of variable generational persistence. We also reject a model in which the Wardell KMP is divided into an eastern and western subpopulation corresponding to the two large tracts of remnant schlerophyll woodland and forest. Both the Northern and Southern subpopulations are transected by the proposed Section 10 upgrade (Figure 1).

3.2 Regional population structure

Analysis of the spatial distribution of FOR amongst regional koala populations indicate that populations inhabiting the former Big Scrub habitat to the west of the Wardell KMP may be derived from multiple source populations (Figure 4). Spatial patterns indicate higher levels of contemporary dispersal between populations in the former Big Scrub habitat and the Wardell KMP (64%), than between outlying regional populations (Far-western 30%; North Lismore 6%). Spatial patterns also indicate variable levels of contemporary dispersal between populations in the Big Scrub habitat, and the Southern (52%) and Northern (12%) subpopulations of the Wardell KMP. The extent to which these patterns reflect historical patterns of colonisation is, however, uncertain.

Pairwise estimates of genetic differentiation amongst regional populations ranged from 0.03 to 0.071 (*Rst*) and 0.027 to 0.099 (*Fst*) in comparisons with the Wardell KMP subpopulations (Table 4; SCU unpublished data). Both estimators indicate that the Northern and Southern subpopulations of the Wardell KMP are genetically differentiated (*Rst* = 0.081, *Fst* = 0.058) and share closer genetic affinities with populations inhabiting the former Big Scrub habitat than each other.

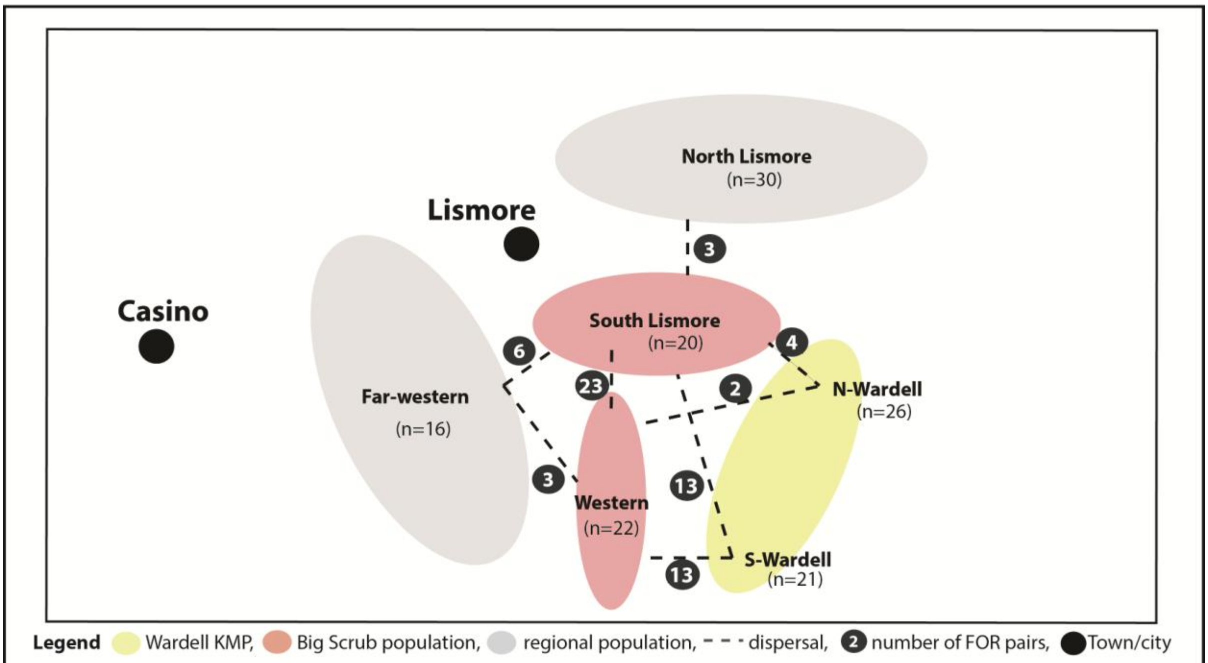

Figure 4. Patterns of dispersal amongst regional koala populations.

Table 4. Genetic differentiation between subpopulations of the Wardell KMP and regional koala populations (SCU, unpublished data). Fst values greater than zero but less than 0.05 indicate low genetic differentiation; values between 0.05 and 0.15 indicate moderate genetic differentiation.

	Rst		**Fst**	
Populations	**N-Wardell**	**S-Wardell**	**N-Wardell**	**S-Wardell**
N-Wardell	-	0.081	-	0.058
S-Wardell	0.081	-	0.058	-
South Lismore	0.043	0.042	0.034	0.027
Western	0.032	0.030	0.042	0.030
North Lismore	0.031	0.068	0.053	0.099
Far-western	0.050	0.071	0.052	0.062

3.3 Genetic variation

Descriptive statistics for both the Northern and Southern subpopulations of the Wardell KMP, as well as the combined sample are presented in Table 5. Levels of genetic variation, as measured by Ho, are within the range reported for populations in northern NSW (0.47-0.78), central NSW (0.5-0.66) and southeast Queensland (0.39-0.73) but exceed those reported for populations in Victoria (0.38-0.56) (from Lee et al 2012). These comparisons are *indicative only* as the number of microsatellites used to estimate Ho varied across the studies. Both AE and Ho indicate higher levels of genetic variation are present in the northern subpopulation of the Wardell KMP. For both subpopulations the average level of inbreeding is negligible with randomisation tests indicating that the observed values are not statistically different from 0.

Table 5. Summary statistics of genetic variation in the Wardell KMP. AE = unbiased estimator of the effective number of alleles; Ho = average observed heterozygosity; He = average expected heterozygosity; Fi = individual inbreeding coefficient.

Subpopulation	Sample size	AE	Ho	He	Fi
Northern	26	3.6	0.668	0.676	0.012
Southern	21	2.76	0.587	0.587	-0.001
Combined*	47	3.33	0.630	0.654	0.038

Discussion and recommendations

4.1 Discussion

This study has shown that the Wardell KMP is spatially structured. Dispersal, estimated from genetic neighbourhood size and the distribution of first-order relatives, largely conforms to an isolation-by-distance model with adjacent areas more likely to share close relatives than distant ones. Of particular relevance, koala dispersal was detected between habitats bisected by the conditionally-approved Section 10 Pacific Highway upgrade in the vicinity of Bagotville, an area previously identified as containing important habitat for dispersal and the main focus of proposed mitigation efforts (Phillips & Chang 2013, Ecosure 2014). Koala dispersal was also detected between habitats in the vicinity of Wardell and Meerschaum Vale suggesting possible dispersal across the valley floor in an area also bisected by the conditionally-approved Section 10 upgrade. These dispersal events were detected with relatively small sample sizes for each location (Table 1) suggesting that dispersal amongst adjacent sites is relatively common.

There is strong evidence that the Wardell KMP, as currently defined, is comprised of a distinctive Northern and Southern subpopulation with detectable level of dispersal between them. The extent of genetic differentiation between them exceeds that observed between populations in the adjacent Big Scrub habitat south of Lismore and outlying regional populations. This is unexpected given their close geographic proximity and evidence of ongoing dispersal between them. The most likely explanation is that the Northern subpopulation receives immigrant koalas from surrounding populations. This is supported by evidence of higher levels of genetic variation in the Northern subpopulation, measured from both observed heterozygosity and the effective number of alleles (Table 2), with 10 of the 14 surveyed microsatellites containing a greater diversity of repeat lengths than observed in the Southern subpopulation. Immigration of koalas into the Northern subpopulation is also supported by significant differences in the average relatedness (R) of males and females in the two subpopulations. This was especially pronounced for females with those in the Northern subpopulation having fewer FOR relatives living in close geographic proximity. This is unexpected for a species in which dispersal away from the natal site is limited.

In contrast, the genetic and demographic profile of the Southern subpopulation appears to be consistent with that expected in a functional koala metapopulation. This includes a high proportion of FOR occurring in close geographic proximity, a decrease in relatedness with distance (isolation-by-distance effects), an absence of detectable inbreeding effects and females, on average, being more closely related than males consistent with a known sex-bias in dispersal rates (Dique et al. 2003) and the structuring of koala populations along matriarchal lines.

It is unclear from the present analysis whether the Northern subpopulation is part of the Wardell KMP historically but has received higher levels of immigration from surrounding areas, or if it has been recently colonised along with other areas of the former Big Scrub habitat south of Lismore. A third possibility is that the boundary of the Wardell KMP, as currently defined, does not accurately reflect the natural (historical) population boundary and the inclusion of samples from peripheral populations of mixed origins has biased the analysis. Unfortunately, current sample sizes were insufficient to enable a more detailed analysis of this issue. Irrespective of the historical processes, these results emphasise the potential significance of the Southern subpopulation of the Wardell KMP as the remaining relatively pure gene pool for koalas in this region.

Spatial patterns of dispersal indicate that koalas inhabiting areas of the former Big Scrub rainforest to the west of the Wardell KMP are derived from multiple source populations including the Wardell KMP and those to the north and west of Lismore. Initial assessments indicate that the greatest number of dispersal events occur between the Southern Wardell subpopulation and those in the former Big Scrub rainforest habitat. Dispersal is most likely asymmetric, occurring predominantly from the Southern Wardell subpopulation into the former Big Scrub habitat. This is supported by the observation of lower levels of genetic variation in the Southern subpopulation compared to the Northern subpopulation (Table 5), as well as regional populations, especially South Lismore (SCU, unpublished data). If substantial levels of immigration were occurring into the Southern subpopulation of the Wardell KMP we would expect more uniform levels of genetic variability across all populations in the region. Asymmetric dispersal is most likely influenced by a combination of demographic, behavioural and landscape factors (e.g., density-dependent regulation, territioriality and the spatio-temporal configuration of dispersal corridors) and a greater understanding of these is required to fully discern the role of the Wardell KMP in regional koala population dynamics.

4.2 Limitations

- Spatial patterns in the distribution of FOR have been used to identify koala dispersal *events* within the Wardell KMP, and between regional koala populations, but we cannot infer dispersal *routes* or directly infer the *direction* of dispersal in most cases.
- It is beyond the scope of this study to discern the factors that facilitate long-distance dispersal events across the Wardell KMP or proposed asymmetric dispersal between regional populations. Demographic, behavioural and landscape factors are likely to play a role.
- Due to limited sample sizes the inclusion of the central cluster (Wardell and Meerschaum Vale) in the Northern Wardell subpopulation is considered provisional only.

4.3 Recommendations

To model the impacts of the conditionally-approved Section 10 Pacific Highway upgrade on the Wardell KMP, and the effectiveness of proposed mitigation strategies, the PVA should incorporate information from this study with respect to the presence of population substructure within the Wardell KMP, potential impacts from disrupting observed patterns of dispersal, and relationships with regional koala populations.

Specifically, the PVA should;

- Be conducted under different assumptions of population structure within the Wardell KMP; a null model with the Wardell KMP treated as a single panmictic population and compared to an alternative model with isolation-by-distance effects incorporated, and a model in which the Wardell KMP is represented by a distinctive Northern and Southern subpopulation as defined here.
- Examine the effects of disrupting patterns of dispersal in the Bagotville area and across the valley floor and adjacent woodland in the vicinity of Wardell and Meerschaum Vale.

- Consider the effects of regional koala population dynamics on the viability of the Wardell KMP in which dispersal approximates a model with net emigration from the Southern Wardell subpopulation (into adjacent habitats to the west), and net immigration into the Northern Wardell subpopulation (most likely from the west and/or north).

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Appendices

Genetic profiling of koalas:
Woolgoolga to Ballina Pacific Highway Upgrade
(Section 10–Wardell to Coolgardie)

Southern Cross University

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October 2015

Executive summary

These Appendices detail additional analyses and information requested by NSW Roads and Maritime Services as a supplement to the report *Genetic profiling of koalas: Woolgoolga to Ballina Pacific Highway Upgrade (Section 10-Wardell to Coolgardie)* (Norman et al 2015) undertaken by Southern Cross University (SCU). The analyses were commissioned in response to comments obtained during the external review process of this report and comparisons with a parallel report submitted by the Australian Museum Research Institute (Neaves et al 2015). These analyses aim to provide some standardisation of the information content of the two reports. Key findings are summarised below.

Spatial autocorrelation, principal component analysis (PCA) and Mantel tests all provide evidence of isolation-by-distance effects within the Wardell KMP.

Model-based clustering using PARTITION-ML confirmed the presence of two regional populations; one located to the north of Lismore, the other encompassing areas to the south and west of Lismore including the WKMP.

Assignment tests and model-based clustering indicate some movement of animals between these two regional populations is likely.

These results support the findings of our initial report (Norman et al. 2015) that there is fine-scale genetic structure with the WKMP and weak but detectable genetic differentiation at larger spatial scales. These effects reflect asymmetric levels and patterns of dispersal across the region and should be accounted for in the accompanying population viability analysis (PVA) of the WKMP.

Contents

Genetic diversity data for the Wardell Koala Metapopulation

These Appendices detail additional genetic diversity data for the 14 microsatellite loci used to estimate the Coefficient of Relatedness (R), Rousett's distance (*Aij*) and F-statistics (*Fst* and *Rst*) for animals in the Wardell Koala Metapopulation (Norman et al. 2015). Primer sequences and details of the 14 loci examined were sourced from the literature: Phc11, Phc13, Phc25 (Houlden et al. 1996a); K2.1, K10.1, Pcv2, Pcv6.1, Pcv25.1, Pcv25.2, Pcv30, Pcv31 (Cristescu et al. 2009); Phci5, Phci9, Phci15 (Ruiz-Rodriguez et al. 2014).

Appendix A provides information on allele frequency distributions for the 14 loci with data presented for the combined WKMP sample as well as the Northern and Southern subpopulations. As outlined in our accompanying report (Norman et al. 2015) there are marked differences in allele frequency distributions with the Northern subpopulation having a greater number of alleles at most loci. We concluded that this was most likely due to higher levels of immigration (or dispersal) of koalas from areas to the north and west leading to greater genetic diversity in the Northern subpopulation (Norman et al. 2015). This is supported by the results of our PARTITION-ML analysis detailed at Appendix I.

Appendix B provides more detailed results of the tests we conducted to evaluate Hardy-Weinberg equilibrium (HWE) (summarised on p.5; Norman et al 2015). Departures from HWE indicate the possible presence of selection at a locus, or can occur as a result of population admixture, non-random mating or population substructure (Wahlund effects). We used GENEPOP 4 to test for heterozygote deficiencies (estimated as Fis, the inbreeding coefficient) (Weir & Cockerham 1984) with significance evaluated using a Markov chain randomisation procedure. Significant departures from HWE were detected at three loci (K10.1, Pcv30 and Phci5) in the Northern subpopulation and are most likely a consequence of non-random mating and population admixture associated with the immigration or dispersal of koalas from surrounding areas.

Appendix C provides results of several tests we performed to check for null alleles. Null alleles result from the failure of one allele to amplify and may lead to errors in genotyping. The presence of null alleles is inferred where there is a deficit of heterozygotes or evidence of large allele dropout, however, heterozygote deficits can also result from non-random population processes (see above).

We first used MICROCHECKER to test for null alleles and genotyping errors due to stutter bands. To minimise errors arising from stuttering all reverse primers used in this study were

PIG-tailed (Brownstein et al 1996) with a seven base pair extension (GTTTCTT). MICROCHECKER did not detect evidence of null alleles or genotyping errors at any locus.

As our earlier tests of HWE identified heterozygote deficits at three loci we also employed a maximum likelihood test for the presence of null alleles performed using ML-Null with null frequencies calculated using GENEPOP 4. This analysis indicated the presence of null alleles in the Northern subpopulation at the three loci exhibiting heterozygote deficiencies (K10.1, Pcv30 and Phci5). Additional analyses presented here and in our report (Norman et al. 2015) indicate that observed heterozygote deficits are more likely to be the result of non-random population processes than PCR artefacts. Consequently, genotyping errors resulting from the presence of null alleles are considered to be negligible or absent in this dataset.

Appendix D provides results of tests for linkage disequilibrium amongst the 14 microsatellite loci performed using GENEPOP 4 with Bonferonni correction for multiple tests. There was no evidence of LD in the Southern Wardell subpopulation although LD was detected for two pairs of loci in both the Northern Wardell subpopulation and the combined WKMP sample. As patterns of LD were not consistent across populations it is most likely that the observed disequilibrium has arisen through population-specific processes rather than physical linkage of the loci on a chromosome. LD can arise through genetic drift and is not unexpected in small populations or where inbreeding occurs. Immigration can also lead to increased levels of LD where the source populations are genetically differentiated and is the most likely explanation for the LD observed in the Northern Wardell subpopulation of the WKMP. Population-specific processes are also supported by the absence of LD detected in other studies employing these loci (Cristescu et al. 2009, Lee et al. 2010. Lee et al. 2012, Ruiz-Rodriguez et al. 2014). Conversely, Houlden et al. (1996b) found LD at multiple loci but was able to attribute this to the effects of genetic drift after partitioning the variance components of the disequilibrium coefficients within and between populations. Thus, the detection of LD at a small number of loci in the present study is unremarkable.

Appendix E presents several estimators of genetic diversity (± SE) for the WKMP calculated using GENALEX.

Appendix A. Microsatellite allele sizes, repeat number and frequency data for the combined WKMP samples and the Northern and Southern subpopulations

	Alleles		Frequencies		
Locus	**Size**	**Repeats**	**WKMP**	**Northern**	**Southern**
Phc11	155	15	0.01	-	0.03
	157	16	0.21	0.22	0.20
	159	17	0.07	0.13	-
	161	18	0.05	0.04	0.08
	165	20	0.05	0.09	-
	169	22	0.01	0.02	-
	177	26	0.01	0.02	-
	179	27	0.17	0.11	0.25
	181	28	0.35	0.30	0.43
	183	29	0.05	0.07	0.03
Phc13	115	23	0.03	0.06	-
	117	24	0.14	0.11	0.18
	119	25	0.49	0.39	0.63
	121	26	0.12	0.19	0.03
	123	27	0.13	0.11	0.15
	125	28	0.05	0.07	0.03
	131	31	0.02	0.04	-
	133	32	0.01	0.02	-
	135	33	0.01	0.02	-
Phc25	125	31	0.53	0.50	0.58
	129	33	0.07	0.07	0.08
	135	36	0.01	0.02	-
	137	37	0.02	0.04	-
	147	42	0.16	0.19	0.13
	151	44	0.05	0.06	0.05
	153	45	0.13	0.09	0.18
	155	46	0.02	0.04	-
K2.1	149	13	0.05	0.02	0.10
	155	16	0.19	0.28	0.08
	159	18	0.27	0.31	0.20
	161	19	0.01	0.02	-
	163	20	0.07	0.09	0.05
	173	25	0.36	0.24	0.53
	175	26	0.04	0.04	0.05
K10.1	126	10	0.17	0.06	0.33
	128	11	0.38	0.31	0.48
	130	12	0.03	0.06	-
	132	13	0.11	0.17	0.03
	138	16	0.01	-	0.03
	140	17	0.02	0.04	-
	142	18	0.01	0.02	-
	146	20	0.10	0.09	0.10
	152	23	0.06	0.09	0.03
	154	24	0.11	0.17	0.03

	Alleles		Frequencies		
Locus	**Size**	**Repeats**	**WKMP**	**Northern**	**Southern**
Pcv2	121	14	0.01	0.02	-
	131	19	0.27	0.11	0.28
	133	20	0.28	0.26	0.30
	135	21	0.32	0.39	0.23
	137	22	0.12	0.20	-
	139	23	0.01	0.02	-
Pcv6.1	211	17	0.05	0.09	-
	213	18	0.02	0.04	-
	215	19	0.01	0.02	-
	217	20	0.20	0.28	0.10
	221	22	0.20	0.15	0.28
	223	23	0.23	0.30	0.15
	225	24	0.28	0.13	0.48
Pcv25.1	72	1	0.01	-	0.03
	74	2	0.72	0.67	0.80
	82	6	0.05	0.06	0.05
	88	9	0.07	0.11	0.03
	90	10	0.11	0.11	0.10
	94	12	0.03	0.06	-
Pcv25.2	172	17	0.85	0.81	0.90
	174	18	0.14	0.17	0.10
	178	20	0.01	0.02	-
Pcv30	183	47	0.01	0.02	-
	195	53	0.45	0.39	0.53
	197	54	0.13	0.30	0.03
	199	55	0.38	0.33	0.45
	201	56	0.02	0.04	-
	203	57	0.01	0.02	-
Pcv31	213	14	0.40	0.54	0.23
	223	19	0.18	0.17	0.20
	233	24	0.19	0.04	0.40
	239	27	0.22	0.26	0.18
Phci5	146	11	0.09	0.02	0.19
	150	12	0.01	0.02	-
	154	13	0.08	0.06	0.11
	158	14	0.14	0.13	0.17
	162	15	0.19	0.20	0.17
	166	16	0.49	0.57	0.36
Phci9	164	15	0.65	0.61	0.70
	167	16	0.14	0.15	0.13
	170	17	0.20	0.22	0.18
	173	18	0.01	0.02	-
Phci15	208	10	0.10	0.06	0.15
	212	11	0.01	0.02	-
	216	12	0.53	0.41	0.70
	220	13	0.35	0.50	0.15
	224	14	0.01	0.02	-

Appendix B. Tests of Hardy-Weinberg equilibrium for the combined WKMP samples and the Northern and Southern subpopulations. *, denotes loci showing significant departures from HWE with a deficit of heterozygotes (exact P-value ≤ 0.05).

	WKMP combined		Northern		Southern	
Locus	**Fis**	**P-value**	**Fis**	**P-value**	**Fis**	**P-value**
Phc11	-0.123	0.95	-0.159	0.90	-0.101	0.84
Phc13	0.017	0.14	-0.027	0.17	0.035	0.69
Phc25	0.020	0.09	-0.046	0.10	0.131	0.17
K2.1	0.105	0.36	0.038	0.35	0.121	0.38
K10.1	0.091	0.06	0.071	0.03*	0.035	0.53
Pcv2	0.116	0.15	0.000	0.37	0.157	0.29
Pcv6.1	0.115	0.12	0.170	0.06	-0.100	0.56
Pcv25.1	0.031	0.32	0.031	0.41	0.015	0.58
Pcv25.2	0.015	0.62	0.057	0.55	-0.086	1.00
Pcv30	0.075	0.00*	0.112	0.01*	-0.029	0.65
Pcv31	0.002	0.39	-0.186	0.94	0.052	0.45
Phci5	0.046	0.12	0.106	0.03*	-0.063	0.58
Phci9	-0.179	0.98	-0.183	0.96	-0.161	0.91
Phci15	0.137	0.27	0.125	0.28	-0.049	0.73

Appendix C. Estimated null allele frequencies and P-values for the 14 microsatellite loci. Tests were computed for the combined WKMP sample and the Northern and Southern subpopulations using GENEPOP 4 (null frequencies per locus) and ML-Null (randomisation tests). *, denotes loci showing a significant deficit of heterozygotes (exact P-value ≤ 0.05) and the possible presence of null alleles. As we have shown that these heterozygote deficits are most likely due to non-random population processes we have not computed corrected allele frequency distributions.

	WKMP combined		Northern		Southern	
Locus	**Null Freq.**	**P-value**	**Null Freq.**	**P-value**	**Null freq.**	**P-value**
Phc11	0	0.95	0	0.90	0	0.83
Phc13	0.019	0.15	0.216	0.17	0	0.65
Phc25	0.042	0.10	0.047	0.09	0.044	0.15
K2.1	0.004	0.34	0	0.37	0	0.37
K10.1	0.153	0.56	0.203	0.02*	0	0.46
Pcv2	0.036	0.15	0	0.36	0	0.28
Pcv6.1	0.038	0.12	0.063	0.06	0	0.54
Pcv25.1	0.011	0.29	0.019	0.41	0	0.25
Pcv25.2	0.006	0.36	0.021	0.55	0	0.15
Pcv30	0.057	<0.01*	0.072	<0.01*	0	0.50
Pcv31	0.004	0.39	0	0.94	0	0.41
Phci5	0.027	0.09	0.05	0.03*	0	0.47
Phci9	0	0.98	0	0.96	0	0.73
Phci15	0.038	0.25	0.03	0.28	0	0.60

Appendix D. Tests of linkage disequilibrium linkage for the 14 microsatellite loci after Bonferroni correction (significant LD if P ≤ 0.0036). No LD was observed amongst loci in the Southern Wardell subpopulation.

WKMP			Northern			Southern		
Locus1	**Locus2**	**P-value**	**Locus1**	**Locus2**	**P-value**	**Locus1**	**Locus2**	**P-value**
Phc11	Pcv6.1	0.002	Pcv30	Pcv31	0.003	-	-	-
Pcv25.2	Phci15	<0.001	Pcv25.2	Phci15	0.003	-	-	-

Appendix E. Genetic diversity estimates averaged over the 14 microsatellite loci in the WKMP. Statistics were calculated for the Northern and Southern subpopulations as well as the combined WKMP sample. *n*, sample size; *Na*, number of alleles; *Ne*, effective number of alleles, *Pa*, private alleles, *Ho*, observed heterozygosity; *He*, expected heterozygosity; *F*, fixation index; SE, standard error.

Sub-population	***n***	***Na* ± SE**	***Ne* ± SE**	***Pa* ± SE**	***Ho* ± SE**	***He* ± SE**	***F* ± SE**
Northern	27	6.29 ± 0.5	3.44 ± 0.3	2.14 ± 0.3	0.67 ± 0.04	0.66 ± 0.04	-0.01 ±0.03
Southern	20	4.29 ± 0.4	2.60 ± 0.2	0.14 ± 0.1	0.58 ± 0.04	0.57 ± 0.04	-0.03 ± 0.02
Combined	47	6.43 ± 0.6	3.26 ± 0.3	6.43 ± 0.6	0.63 ± 0.04	0.65 ± 0.04	0.02 ± 0.02

Population genetic analysis of the Wardell Koala Metapopulation

Appendices F-H provide details of additional population genetic analyses of the WKMP. The first (Appendix F) provides two estimates of the effective population size (N_e) of the combined WKMP sample and the two subpopulations (Northern and Southern) calculated using the program Ne ESTIMATOR. The first uses the linkage disequilibrium method and assumes random mating while the second employs the molecular co-ancestry method which is appropriate for population samples comprising a single cohort. As our data does not meet the assumptions of either model we urge caution in interpreting these outputs. Furthermore, estimates of N_e cannot be compared directly with estimates of census population size obtained from survey data. The larger estimated N_e for the Northern Wardell subpopulation is consistent with other data presented herein and Norman et al (2015) that immigration/dispersal contributes to higher genetic diversity, heterozygote deficiencies and genetic disequilibrium in this sample.

Appendix G provides the results of a Mantel test for isolation-by-distance (IBD) performed by regressing both (a) Relatedness and (b) genetic distance on geographic distance. A significant relationship (P (rxy random ≥rxy data) was observed for both analyses (P = 0.001 for relatedness, P = 0.01 for genetic distance). This is consistent with our previous analyses in which IBD effects were inferred from estimates of genetic neighbourhood size (21-30 km^2) and spatial patterns in the distribution of First Order Relatives. We found closely related individuals were spatially clustered, occurring within or between adjacent sampling areas within the WKMP. Nevertheless, instances of long-distance dispersal across the WKMP study area were also detected but at a much lower frequency.

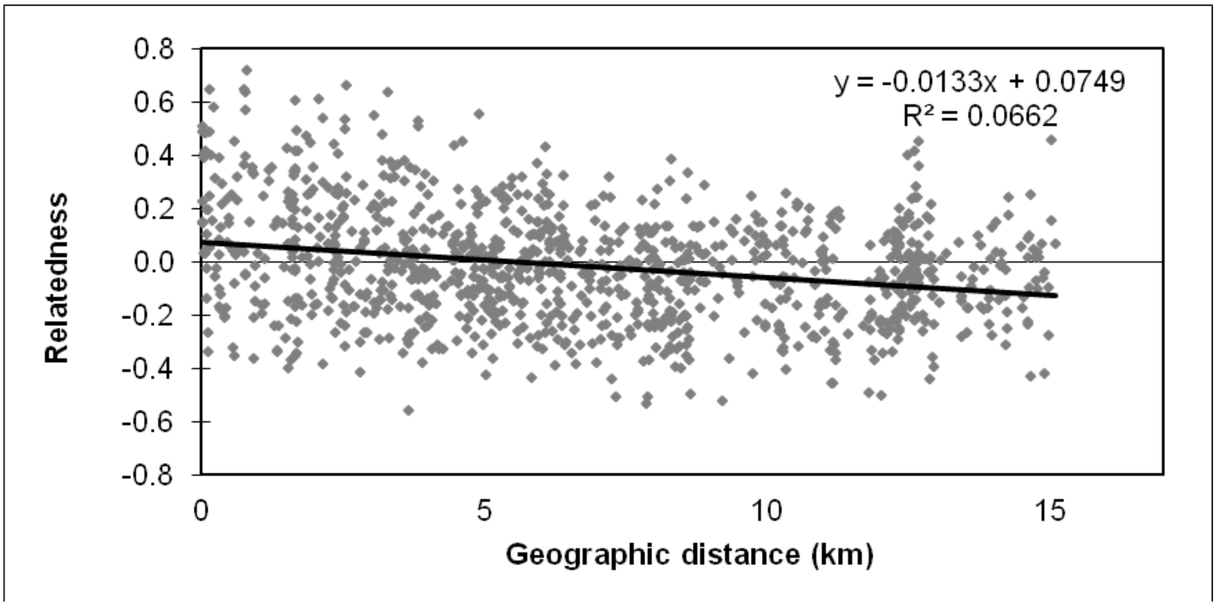
Spatial structuring within the WKMP was also apparent from the spatial autocorrelation analysis (Appendix H) calculated using Nei's genetic distance. The failure of Neaves et al. (2015) to detect spatial structuring using this approach may be a function of the smaller sample size (38 compared with 47) used in their analysis resulting in relatively few comparisons for some distance classes.

Appendix F. Estimates of effective population size (*Ne,* plus 95% confidence intervals) for the WKMP and the two subpopulations. *Ne* was calculated using the linkage disequilibrium model (LD model) with a lower bound allele frequency of 0.02, and the molecular coancestry model (MC model).

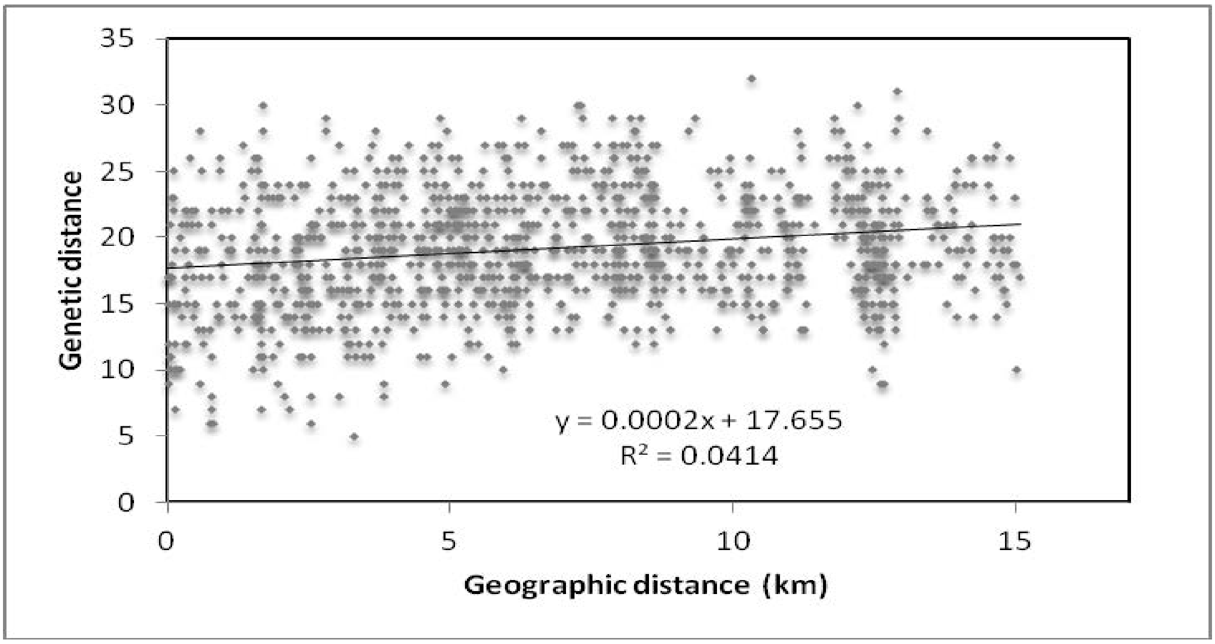
	LD model		MC model	
Population	***Ne***	**95% CI**	***Ne***	**95% CI**
Northern	65.2	38.4 – 168.8	13.7	3.3 – 31.4
Southern	14.9	10.3 – 23.0	8.3	2.5 – 17.5
Combined	47.4	36.8 – 63.3	9.6	5 – 15.7

Appendix G. Mantel tests of isolation-by-distance in the WKMP. **a.** Relationship between Wang's relatedness coefficient (R) and geographic distance. **b.** Relationship between Nei's genetic distance and geographic distance. km, kilometres.

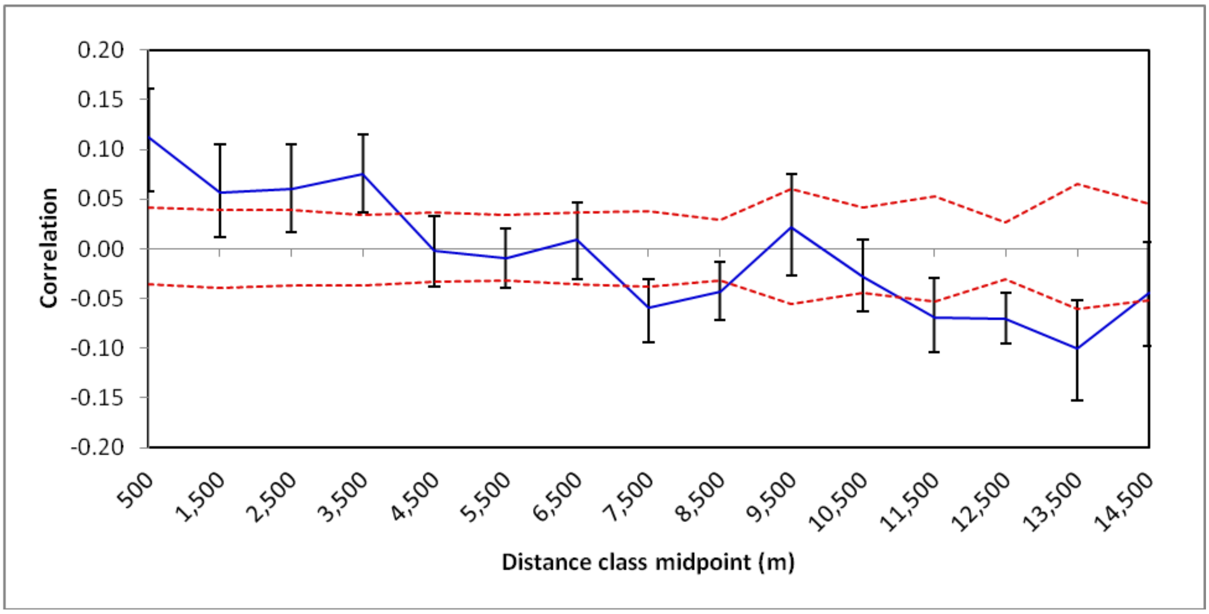
a.



b.



Appendix H. Spatial autocorrelogram for the WKMP. The blue line shows the correlation of average pairwise relatedness (±SE) as a function of geographic distance classes in metres (m). Red dotted lines show the 95% confidence interval around a null hypothesis of no spatial structure.



Genetic analysis of regional koala population structure

These Appendices detail additional analyses of regional koala population structure that are most appropriate for detecting genetic patterns arising through the action of long-term (evolutionary) processes over large spatial scales. These analyses are not suitable for discerning aspects of fine-scale population structure within the WKMP that were required to inform the concurrent Population Viability Analysis.

Appendix I presents results of a model-based clustering analysis of the 134 koala samples from the WKMP, areas of the former Big Scrub rainforest (South Lismore, Western and North Lismore) and habitat to the west of Lismore. These populations have been analysed previously using the program STRUCTURE (Lee et al. 2013) which identified the presence of two populations; one occurring to the north of Lismore and extending into south-east Queensland (QLD), the other occurring to the south and west of Lismore. To complement this analysis we employed an alternative program (PARTITION-ML) which implements a maximum likelihood model to discern population structure. In this analysis samples are assigned to populations with the number of populations (*k*) iterated over the range 1-5 with the resulting likelihoods used to identify the best fit model *via* a Likelihood Ratio test. PARTITION-ML also identified a distinct northern (North Lismore samples) and southern (Wardell, South Lismore, Western and Far-western samples) population. Both studies also found evidence of low level dispersal/immigration between the two regional populations. In our PARTITION-ML analysis eleven (11) koalas sampled to the south of Lismore were assigned to the northern population. Five (5) of these animals were from the Northern Wardell subpopulation, three (3) from the Western population and three (3) from the South Lismore population. No samples from the Southern Wardell subpopulation were identified as being from North Lismore. These results are consistent with our previous analyses that revealed higher genetic diversity in the Northern Wardell subpopulation and Fst/Rst values that aligned it more closely with the North Lismore population.

Recent studies have shown that programs such as STRUCTURE and PARTITION-ML that employ model-based clustering to identify population structure perform poorly when there is weak genetic differentiation (Putman & Carbone 2014) such as that expected to occur at finer spatial scales. The lower limit of detection for STRUCTURE was found to be *Fst* 0.03, although accurate assignment of individuals to populations requires *Fst* to be 0.05 or above (Latch et al. 2006). Given that our previous analyses show levels of population differentiation ranging from *Fst* 0.027-0.062 (Norman et al. 2015) model-based clustering programs are at the lower limits for detecting regional population structure in this dataset and are unsuitable for detecting fine-scale population structure within the WKMP.

Appendix J shows results of the AMOVA analysis for the 134 regional koala samples when assigned to (a) two or (b) six populations. In both analyses the among population component of molecular variance was relatively small (6-7%) with most variance attributable to genetic differences within individuals (90%).

Appendix K presents results of an Assignment test conducted in GENALEX which indicate that 5% of koalas were sampled at sites other than their population of origin. This is consistent with results of other analyses that indicate some immigration/dispersal between the North Lismore population and those to the south (WKMP, South Lismore, Western and Far-western).

Appendix L presents the results of a principal component analysis (PCA) of genetic dissimilarity amongst 134 koalas sampled from the WKMP and surrounding areas. There is evidence of weak genetic structure consistent with IBD effects across the region. Neaves et al. (2015) found a similar pattern of weak genetic structuring amongst populations in northern NSW (Wardell and Tyagarah) and south-east Queensland (Coomera) when analysed using PCA and discriminant analysis of principal components (DAPC, in which these three populations collapsed to a single cluster) with strongly demarcated populations only detected at Port Macquarie and Coffs Harbour to the south. Lee et al. (2013) in an earlier study utilising 6 microsatellite loci also found weak genetic structure amongst koala populations from across this region. Weak genetic structure is expected to arise within and between populations when there are restrictions on the level and direction of gene flow (migration-dispersal) across the landscape. Discerning these patterns to inform the concurrent PVA was the main objective of our report (Norman et al. 2015). Strong genetic differentiation of populations is only likely where there are (i) long-term barriers to dispersal with limited or no genetic exchange amongst populations, and (ii) sufficient time has elapsed for random drift, mutation, selection and non-assortative mating to cause detectable changes in the genetic makeup of the populations.

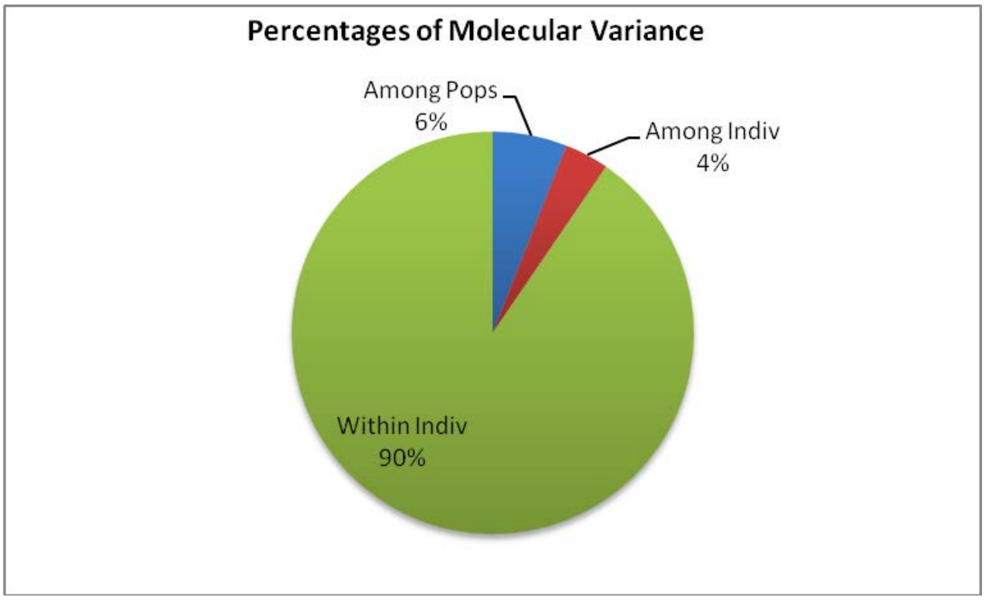
Appendix I. Number of regional populations identified using model-based clustering as implemented by PARTITION-ML. k, number of populations; L, likelihood for the model; df, degrees of freedom; CV, critical value for the chi-squared test (X^2). Significance was tested using the Likelihood Ratio test for k_n+1 with partitions identified as significant at the P = 0.05 level indicated (*).

PARTITION-ML Model Parameters (df = 111, CV = 87.68)		
k	**L**	X^2
1-null model	-3892.43	
2	-3833.54	117.78*
3	-3818.39	30.3
4	-3807.20	22.38
5	-3789.24	35.92

Appendix J. AMOVA partitioning of genetic diversity among and within regional koala populations. **a.** Samples partitioned into two populations based on the results of the PARTITION-ML analysis. **b.** Samples partitioned into six regional populations following Norman et al (2015). For this analysis within population diversity was also partitioned into the among and within individual (ind.) components. **c.** Partitioning of molecular variance for the six regional populations shown graphically.

Source	df	SS	MS	Est. Var.	%
a. Regional-2					
Among pops	1	40.16	40.16	0.32	7
Within pops	266	1213.03	4.56	4.56	93
Total	267	1253.19		4.88	100
b. Regional-6					
Among pops	5	85.70	17.14	0.29	6
Within pops	262	1167.50	4.46	4.46	94
(among ind.)	(128)	(592.50)	(4.63)	(0.17)	(4)
(within ind.)	(134)	(575.00)	(4.29)	(4.29)	(90)
Total	267	1253.19		4.74	100

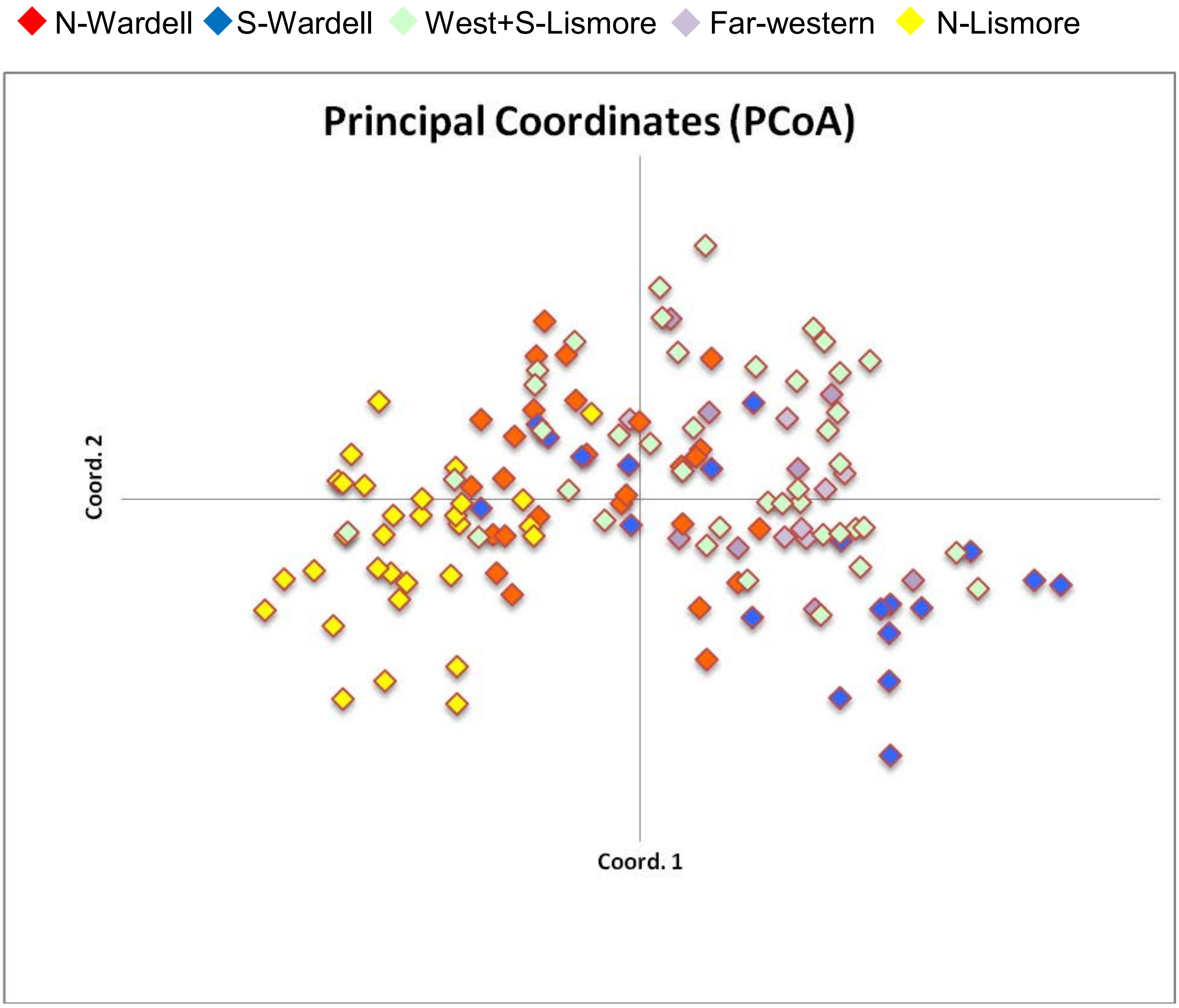
c.



Appendix K. Assignment test for regional koala populations. Samples were assigned to a northern (North Lismore) and southern population (WKMP, South Lismore, Western and Far-western). 95% were detected in their assigned population of origin whereas 5% were sampled outside their assigned population of origin.

Population	Original pop.	Other pop.
Northern	30	1
Southern	97	6
Total	127	7
Percent	95%	5%

Appendix L. Principal component plot of genetic distances between koalas from the WKMP and surrounding regions.



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Supplementary Appendices 1

Genetic profiling of koalas: Woolgoolga to Ballina Pacific Highway Upgrade

(Section 10–Wardell to Coolgardie)

Southern Cross University

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& Prof. L. Christidis

October 2015

Executive summary

Supplementary Appendices S1a-S1d detail additional analyses and information requested by NSW Roads and Maritime Services as a supplement to the report *Genetic profiling of koalas: Woolgoolga to Ballina Pacific Highway Upgrade (Section 10-Wardell to Coolgardie)* (Norman et al. 2015) undertaken by Southern Cross University (SCU), and the Appendices to that report. The analyses herein provide genetic estimates of dispersal, estimated as the number of migrants per generation (*Nm*) calculated using Shannon's Mutual Information Index and assuming symmetrical migration. *Nm* was calculated for;

(i) populations of koalas occupying habitat to the east and west of the conditionally-approved Section 10 Pacific Highway upgrade, and
(ii) between the Wardell Koala Metapopulation (WKMP) and surrounding areas.

Key findings and recommendations are:

The estimated mean migration rate for koalas between habitats to the east and west of the conditionally-approved Section 10 Pacific Highway upgrade ranged from 9.5 to 273 migrants per generation (S1d). Assuming a generation time of 6.02 – 7.8 years it is estimated that between 1.2 and 45 koalas/year migrate between these habitats.

The estimated mean migration rate for koalas between the WKMP and surrounding regional populations ranged from 4.9 to 51 migrants per generation for the Northern population, and 9.9 to 115 for the Southern population (S1d). Assuming a generation time of 6.02 – 7.8 years it is estimated that 0.6 to 8.5 koalas/year migrate between WKMP and the Northern population with migration between WKMP and the Southern population higher at 1.3 to 19.1 koalas/year.

Estimates of *Nm* presented here re likely to be lower than the number of dispersing koalas as migration rates do not account for transient dispersal by koalas which includes sub-adults seeking to establish new territories, or resident koalas traversing their normal home ranges.

Due to numerous sources of variability associated with the estimation of migration rates we advise caution when using *Nm* as input for Population Viability Analysis. We recommend that estimated values of *Nm* presented herein should serve as a guide for the levels of migration to be included in sensitivity analyses for the PVA rather than absolute values of migration.

Contents

Estimation of koala dispersal

These Supplementary Appendices provide estimates of the number of koalas exchanged between regional populations and dispersing across the conditionally-approved Section 10 Pacific Highway upgrade. For the purposes of this report, dispersal is expressed as the number of migrants per generation (*Nm*) where *N* is the effective size of each population and *m* is the proportion of individuals that migrate. *Nm* was calculated using Shannon's Mutual Information Index (Sherwin et al. 2006, Rossetto et al. 2008) as implemented in GENALEX. This approach aims to estimate the number of koalas that are able to disperse across a boundary and breed on both sides. *Nm* is likely to be smaller than the total number of dispersing koalas as it does not account for transient animals or those undertaking foraging movements within their home range.

Supplementary Appendix S1a provides estimates of *Nm* for each of the 14 microsatellite loci and the chi-square significance of the G-test for allele frequency differences at each locus. This analysis assumes an effective population size of 500 for each population, an unrealistic assumption for the WKMP (Appendices to Norman et al. 2015) and regional populations (see S1b below). Thus, for subsequent estimation of migration rates we first calculated effective population sizes (*Ne*) from the data and used these as input into the analysis.

Supplementary Appendix S1b provides details of *Ne* estimated for each population using the Linkage Disequilibrium (LD) and Molecular Coancestry (MC) methods implemented in NE ESTIMATOR. We computed *Ne* for different population partitions to determine how sampling strategies and population assignments might affect subsequent estimates of migration. For the WKMP we partitioned the available samples into those occurring east (E-Wardell) and west (W-Wardell) of the conditionally-approved Section 10 Pacific Highway upgrade. Due to the small number of koalas sampled to the east of the upgrade (n=10), and possible isolation-by-distance effects over larger spatial scales to the west, we chose to calculate *Ne* for the entire western sample (W-Wardell[b], n=37) and a smaller subset (W-Wardell[a], n=15) restricted to those animals sampled in forest fragments immediately to the west of the upgrade in the vicinity of Bagotville, Meerschaum Vale and Coolgardie. We also conducted a hierarchical analysis of regional populations with *Ne* estimated for each of the four (4) regional populations analysed by Norman et al. (2015), a combined Sth. Lismore and Western population, the Southern and Northern population clusters identified in STRUCTURE and PARTITION-ML analyses (Lee et al. 2013, Appendices to Norman et al. 2015) and the combined regional populations (All Regional).

All methods of *Ne* estimation are subject to various forms of error, bias and assumptions that may be violated by real data. For LD it is recommended that the sample size (n) should be equal to the estimated *Ne* for reliable results to be obtained (Russell & Fewster 2009). Over half (0.55) of our populations do not meet this criterion (S1b), including E-Wardell and W-Wardell[a]. Nevertheless, estimates of *Ne* calculated using the LD method were used in subsequent computations to determine koala migration as the MC method assumes samples are taken from a single cohort making this a less appropriate method. Both methods, however, failed to provide reliable estimates of *Ne* for some populations returning a point estimate or upper confidence interval of infinity. For LD, this occurs when the observed value is less than expected due to sampling error. In this case there is no evidence of disequilibria caused by genetic drift due to sampling a finite number of individuals – it can all be explained by sampling error (Do et al. 2014). For the LD method we also used critical values of 0.01 and 0.02 to define the frequency at which rare alleles were excluded from the analysis. This led to substantial variation in *Ne* for some populations (S1b) and is an additional source of imprecision. We used LD estimates of *Ne* using the critical value of 0.01 as input for estimation of *Nm*.

Supplementary Appendix S1c presents *Nm* estimates for the WKMP (east and west of the conditionally-approved Pacific Highway upgrade) and surrounding populations. *Nm* was estimated using empirical values of *Ne* calculated by the LD method (S1b) as input. As reliable estimates of *Ne* could not be calculated for E-Wardell and the Northern population we used the lower CI's as input which were not dissimilar to the point estimates of *Ne* obtained for other populations. As our initial analysis also revealed substantial variation in *Nm* estimates obtained for individual loci (S1a) we applied three cutoff values for ${}^{S}H_{UA}$ (0.0001, 0.001 and 0.01) to provide an objective criteria for eliminating anomalous values. Loci exhibiting heterozygote deficiencies or genetic disequilibrium were retained in the analysis as we have previously shown that these were due to population-specific processes and not general features of those loci (Appendices to Norman et al 2015). Additionally, loci exhibiting heterzygote deficits or disequilibria accounted for only a few of the anomalous *Nm* values determined using the ${}^{S}H_{UA}$ cutoff of 0.01 suggesting that their effect on estimation of *Nm* was negligible.

Our analyses show that *Ne*, sampling design, the assignment of population partitions and choice of ${}^{S}H_{UA}$ can substantially influence estimates of *Nm*. For the WKMP, estimates of koala dispersal across the conditionally-approved Section 10 Pacific Highway upgrade ranged from a minimum estimate of 9.5 to a maximum of 273 with a three-fold difference in *Nm* observed between the two W-Wardell partitions (S1c). Given that the WKMP is estimated to comprise ~200 koalas the upper *Nm* value of 273 calculated using a ${}^{S}H_{UA}$ cutoff of 0.0001 appears to overestimate dispersal. Given these factors, and other sources of imprecision in the estimation of *Nm*, it is recommended that sensitivity analyses employing a range of ecologically plausible *Nm* values be conducted to determine the effect of koala dispersal on the viability of the WKMP. For the purposes of the PVA, our estimates of *Nm* were converted to migrants/year assuming a generation time in koalas of 6.02 or 7.8 years (Phillips 2000) (S1d).

Despite the difficulties inherent in estimating *Nm* the results presented here are consistent with the levels and patterns of dispersal we inferred from the spatial distribution of First-Order Relatives and other analyses (Norman et al. 2015). This includes isolation-by-distance effects, high levels of migration between WKMP and populations to the west (South Lismore and Western), and low but detectable migration between WKMP and the Northern population.

Appendix S1a. Migrants per generation (*Nm* ± SE) calculated for each of the 14 loci assuming an effective population size >500 for each population. A cutoff value of 0.0001 was used for $^{S}H_{UA}$ and a log base=2 transformation applied for diploids. W-Wardell[a] is restricted to those koalas sampled from forest fragments immediately to the west of the conditionally approved Pacific Highway upgrade (n=15). W-Wardell[b] includes all koalas sampled to the west of the upgrade (n=37) including those sampled to the north and northwest in areas of the former Big Scrub rainforest. All other populations are as defined in Norman et al. (2015) and Appendices. *, significance of G-test for allele frequency differences calculated for $^{S}H_{UA}$ where P≤0.05.

Pop1	**Pop2**	**K10.1**	**K2.1**	**Pcv2**	**Pcv25.1**	**Pcv25.2**	**Pcv30**	**Pcv31**	**Pcv6.1**	**Phc11**	**Phc13**	**Phc25**	**Phci15**	**Phci5**	**Phci9**	***Nm* ± SE**
E-Wardell	W-Wardell[a]	1.53	62.10	6.93	361	866	28.25	2.32*	5.19	1.47	4.52	10.77	1.96*	2.73	-	104 ± 69
E-Wardell	W-Wardell[b]	3.36	74.61	9.38	834	1632	10.4	9.83	31.14	2.75	6.34	9.64	8.96	6.91	543	227 ± 127
Nm between Wardell and Southern populations																
Wardell	Sth Lismore	2.97	1.45*	12.54	4.25*	853	5.09*	98.66	0.81*	1.30*	3.10*	0.52*	21.03	6.03	2954	283 ± 214
Wardell	Western	2.14*	6.67	7.70	39.51	156	6.18*	136	0.83*	2.13*	1.34*	0.97*	34.29	2.74*	60.57	33 ± 14
Wardell	Far-western	2.76	0.45*	1.07*	4.42*	536	5.81*	9.96*	0.26*	1.73*	2.52*	0.84*	13.33	1.18*	15.99	43 ± 38
Wardell	S.Lis+West	2.35*	3.65*	10.92	11.29*	221	3.69*	339	0.66*	1.66*	1.82*	0.66*	25.75	5.67*	1762	171 ± 125
Wardell	All Southern	6.06	2.16*	5.07*	13.86*	591	3.21*	199	0.47*	1.72*	3.29*	0.69*	27.15	5.36*	207	76 ± 44
Nm between Wardell and the Northern population																
Wardell	Northern	0.76*	0.89*	1.52*	1.57*	183	2.92*	2.59*	0.24*	3.79*	3.35*	0.11*	1.32*	1.52*	9.01*	15 ± 13
Nm between Wardell and the combined regional populations																
Wardell	Regional	5.86*	3.30*	8.03*	6.96*	1434	8.56*	232	0.51*	6.95*	10.98*	0.54*	31.77*	74.45	141	140 ± 101

Appendix S1b. Effective population size (*Ne*) for koalas in the WKMP and surrounding areas. *Ne* calculated using the linkage disequilibrium (LD) and molecular coancestry (MC) methods with 95% confidence intervals (CI). N, sample size. *, denotes sample sizes that are within 10% of the estimated *Ne*. LD critical values for exclusion of rare alleles in brackets. inf, infinity.

Population	n	LD (0.02)	95% CI	LD (0.01)	95% CI	MC	95% CI
Population partitions and *Ne* for Wardell							
E-Wardell	10	148.0	20.3-inf.	148.0	20.3-inf.	inf.	inf.
W-Wardell[a]	15	21.8	14.9-35.3	21.8	14.9-35.3	inf.	inf.
W-Wardell[b]	37*	37.9	28.8-52.5	20.6	17.1-25.0	12.7	4.4-25.3
Wardell	47*	47.4	36.8-63.6	24.4	20.8-28.9	9.6	5-15.7
Population partitions and *Ne* for regional populations							
S Lismore	19	43.6	24.9-123.0	43.6	24.9-123.0	19.8	0-99.5
Western	23*	19.0	13.7-28.2	19.0	13.7-28.2	2.5	1.4-3.8
S Lis.+Western	42*	73.9	48.8-134.7	41.5	32.4-55.4	4.0	1.8-7.0
Far-western	16	28.6	15.3-92.8	28.6	15.3-92.8	9.0	3.3-17.4
Southern	57	115	74.6-221.8	79.2	58.7-115.6	4.2	2.4-6.4
Northern	31	inf.	193.4- inf.	271.6	98.9- inf.	inf.	inf.
All Regional	88*	49.7	*42.5-58.8*	67	*56.8-80.3*	27.2	7.4-59.7

Appendix S1c. Migrants per generation (*Nm*) calculated for each of the 14 loci using empirical estimates of effective population size (*Ne*) calculated by the LD method. Cutoff values for $^{S}H_{UA}$ used in the estimation of *Nm* were set at 0.0001[A] (dash), 0.001[B] and 0.01[C], with those loci falling below this value omitted from the calculation of mean *Nm*. W-Wardell[a] is restricted to those koalas sampled from forest fragments immediately to the west of the conditionally approved Pacific Highway upgrade (n=15). W-Wardell[b] includes all koalas sampled to the west of the upgrade (n=37) including those sampled to the north and northwest in areas of the former Big Scrub rainforest. All other populations are as defined in Norman et al. (2015) and Appendices.

Pop1	Pop2	K10.1	K2.1	Pcv2	Pcv25.1	Pcv25.2	Pcv30	Pcv31	Pcv6.1	Phc11	Phc13	Phc25	Phci15	Phci5	Phci9	[A]*Nm* ± SE	[B]*Nm* ± SE	[C]*Nm* ± SE
Nm between east (E-) and west (W-) Wardell																		
E-Wardell	W-Wardell[a]	1.27	47.33	4.72	287[C]	675[C]	28.64	1.57	4.97	1.01	4.14	6.77	1.63	2.02	-	82 ± 54	82 ± 54	9.5 ± 4.5
E-Wardell	W-Wardell[b]	4.15	152	13.38	2085[C]	909[C]	20.75	16.75	60.87	2.82	10.04	7.30	27.40	12.64	505[C]	273 ± 156	273 ± 156	29.8 ± 13.1
Nm between Wardell and Southern populations																		
Wardell	Sth Lismore	1.72	1.40	10.65	2.54	430[C]	2.54	69.42	0.72	0.83	2.32	0.41	19.24	3.81	2755[C]	235 ± 196	235 ± 196	9.6 ± 5.7
Wardell	Western	1.50	5.37	5.67	27.29	110	4.01	101	0.63	1.55	0.99	0.74	25.39	2.01	45.04	23.7 ± 9.9	23.7 ± 9.9	23.7 ± 9.9
Wardell	Farwestern	1.31	0.26	0.38	2.55	239	2.38	5.37	0.18	0.70	1.11	0.49	4.98	0.51	8.91	19.2 ± 16.9	19.2 ± 16.9	19.2 ± 16.9
Wardell	S.Lis+West	2.25	4.67	12.18	11.36	210	3.25	382[C]	0.74	1.72	1.98	0.74	29.22	5.41	2027[C]	192 ± 144	192 ± 144	23.6 ± 17.1
Wardell	Southern	8.78	4.71	8.82	20.35	704[C]	3.95	390[C]	0.88	2.55	5.43	1.21	44.99	7.26	410[C]	115 ± 59	115 ± 59	9.9 ± 3.7
Nm between Wardell and the Northern population																		
Wardell	Northern	1.30	1.97	2.75	3.26	645[C]	5.93	4.31	0.47	5.46	6.87	0.32	3.59	3.67	23.21	51 ± 46	51 ± 46	4.9 ± 1.6
Nm between Wardell and the combined regional populations																		
Wardell	Regional	7.40	4.71	10.67	8.83	2088[C]	11.06	326[C]	0.69	8.22	14.26	0.74	47.06	103	199	202 ± 147	202 ± 147	34.6 ± 17.1

Appendix S1d. Estimated dispersal between koala populations. Point estimates of *Ne* (*Ne*-Pop1/*Ne*-Pop2) calculated using the LD method were used as input except those values marked with an asterisk (*) which could not be reliably estimated and the lower CI was used. Mean *Nm* is given as migrants per generation calculated using $^{S}H_{UA}$ cutoff values of 0.0001 and 0.001. Migrants/generation is converted to migrants/year assuming a generation time for koalas of 6.02 or 7.8 years.

			***Nm* ± SE** ($^{S}H_{UA}$ cutoff 0.0001)			***Nm* ± SE** ($^{S}H_{UA}$ cutoff 0.01)		
Pop1	**Pop2**	***Ne***	**(per gen.)**	**(6.02 y/gen.)**	**(7.8 y/gen.)**	**(per gen.)**	**(6.02y/gen.)**	**(7.8 y/gen.)**
Mean *Nm* between east (E-) and west (W-) Wardell								
E-Wardell	W-Wardell[a]	20*/22	82 ± 54	13.6 ± 9	10.5 ± 6.9	9.5 ± 4.5	1.6 ± 0.7	1.2 ± 0.6
E-Wardell	W-Wardell[b]	20*/21	273 ± 156	45 ± 26	35 ± 20	29.8 ± 13.1	5 ± 2.2	3.8 ± 1.7
Mean *Nm* between Wardell and Southern populations								
Wardell	S-Lismore	24/43	235 ± 196	39 ± 32	30 ± 25	9.6 ± 5.7	1.6 ± 0.9	1.2 ± 0.7
Wardell	Western	24/19	23.7 ± 9.9	3.9 ± 1.6	3.0 ± 1.3	23.7 ± 9.9	3.9 ± 1.6	3.0 ± 1.3
Wardell	Far-western	24/29	19.2 ± 16.9	3.2 ± 2.8	2.5 ± 2.2	19.2 ± 16.9	3.2 ± 2.8	2.5 ± 2.2
Wardell	S-Lismore+Western	24/42	192 ± 144	32 ± 24	24.6 ± 18.5	23.6 ± 17.1	3.9 ± 2.8	3.0 ± 2.2
Wardell	Southern	24/79	115 ± 59	19.1 ± 9.8	14.7 ± 7.6	9.9 ± 3.7	1.6 ± 0.6	1.3 ± 0.5
Mean *Nm* between Wardell and the Northern population								
Wardell	Northern	24/99*	51 ± 46	8.5 ± 7.6	6.5 ± 5.9	4.9 ± 1.6	0.8 ± 0.3	0.6 ± 0.2
Mean *Nm* between Wardell and the combined regional populations								
Wardell	All regional	24/67	202 ± 147	34 ± 24	26 ± 19	34.6 ± 17.1	5.7 ± 2.8	4.4 ± 2.2

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PEER REVIEW OF TWO REPORTS:

"AMRI"

"Koala population genetics management. A report to the Roads and Maritime Service (RMS)" 12th August 2015. Linda E. Neaves, Siobhan B. Dennison,, Greta J. Frankham, Mark D. B. Eldridge and Rebecca N. Johnson. Australian Museum Research Institute

and

"SCU"

"Genetic profiling of koalas: Woolgoolga to Ballina Pacific Highway Upgrade (Section 10-Wardell to Coolgardie)". August 2015. Dr J.A. Norman, Dr C. Blackmore, Assoc. Prof. R. Goldingay & Prof L. Christidis. Southern Cross University.

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SUMMARY and RECOMMENDATIONS

In the Wardell area of northern NSW, a planned upgrade to the Pacific Highway passes through an area inhabited by koalas. NSW Roads and Maritime Services wishes to assess the possible impact of the upgrade on the koala population, and have commissioned two genetic reports: AMRI (by Australian Museum Research Institute) and SCU (by Southern Cross University).

The question “Could the upgrade disrupt koala dispersal sufficiently to reduce the viability of the koala population?” is expressed as a series of deliverables, plus the apparent intention to follow up with a Population Viability Analysis (PVA). I presume that PVA will be used comparatively, examining chance of population persistence over multiple generations, with various levels of dispersal between sub-populations. Such comparative use of PVA is called “sensitivity analysis”.

To set the baseline for such a PVA, genetic methods can be used to assess the natural amount of dispersal between areas. The margins of areas to be investigated can be set for various reasons including habitat suitability or human alteration (eg the highway upgrade).

AMRI and SCU each used a number of different methods to assess genetic subdivision, without converting them to dispersal estimates. It is currently not possible to make a direct comparison between the results of AMRI and SCU at Wardell, because there is insufficient corresponding geographic information. This should be clarified with detailed geographic information for each individual at Wardell and immediate surrounds. However, in both studies, there was a general pattern of increasing genetic similarity at decreasing separation (“Isolation by Disatnce”), but there were no clear boundaries where one locality was genetically isolated from another.

But what is the dispersal across these boundaries? In each report only one method specifically targeted dispersal, by identifying particular koalas who might have dispersed in the most recent generation: those that were genetically assigned to a location other than the one in which they were sampled (AMRI); or those with first-order relatives in a location other than the one in which they were sampled (SCU). Only a small number of individuals were identified as potential dispersers, but it is worth noting that only small numbers are needed to halt genetic differentiation by chance processes in transmission. Thus the areas within Wardell, appear to be important for mutual support of each other, and adjacent areas.

Both studies indicated that dispersal was relatively high both between subpopulations within Wardell, and between Wardell and adjacent areas. This dispersal probably does two things: opposes loss of genetic variation within subpopulations, and forestalls immediate extinction. Therefore, a precautionary conservation management approach would be to avoid any reduction of the measured level of dispersal, pending results of the PVA-sensitivity analysis.

There are two limitations to the use of AMRI’s and SCU’s genetic dispersal estimates as base-line dispersal for the PVA-sensitivity analysis. The first limitation is that sample sizes are unavoidably small, so that the estimates are subject to high error rates. This is compounded by the fact that these particular analyses only tell us about dispersal in the most recent generation. AMRI and SCU were correct to avoid methods that convert their genetic subdivision estimates into dispersal rates, because this has been criticised on various grounds.

However, there exists a Mutual Information method that avoids the problems that beset other genetic dispersal measures, and can deal with the widest possible range of population sizes and dispersal rates. The data from AMRI and SCU should be used in this way, to produce dispersal assessments as a baseline in the PVA-sensitivity analysis, to investigate how the koala population’s viability might be affected if the Pacific Highway upgrade reduces dispersal below this baseline.

AMRI and SCU also produce estimates of Genetic variation within subpopulations, which can be included in some PVA programs.

Finally, in commissioning the analysis of sensitivity of population extinction to altered dispersal, I encourage the Roads and Maritime Services to require information on not only the most likely outcome, but also the worst-case outcome, to facilitate precautionary management.

GENERAL COMMENTS

In the Wardell area of northern NSW, a planned upgrade to the Pacific Highway passes through an area inhabited by koalas. NSW Roads and Maritime Services wishes to assess the possible impact of the upgrade on the koala population, and have commissioned two genetic reports: AMRI (by Australian Museum Research Institute) and SCU (by Southern Cross University).

The scope of the two reports appears to be slightly different. The question "Could the upgrade disrupt koala dispersal sufficiently to reduce the viability of the koala population?" is expressed as deliverables that are listed by AMRI and SCU. These deliverables are abbreviated as follows.

1. CONNECTIVITY WITHIN WARDELL. AMRI 1: Analyses of population structure and gene flow within the focal area. Also SCU 1: Is the Wardell KMP spatially structured?

2. CONNECTIVITY BETWEEN WARDELL AND SURROUNDS. AMRI 2: Determine whether the focal population appears to be connected to populations in the surrounding area. Also SCU 2: Is the Wardell KMP an important source population for surrounding areas?

3. WITHIN-LOCALITY GENETIC VARIATION. AMRI 3. Allele frequency information for the population as a whole (and for each subpopulation if relevant).

4. VALIDATION. AMRI 4. Summary of procedures undertaken for data validation.

The SCU report indicated the intention to follow these reports with a formal Population Viability Analysis (PVA). I presume that PVA will be used comparatively, examining chance of population persistence over multiple generations, with various levels of dispersal between sub-populations. Such comparative use of PVA is called "sensitivity analysis" (Penn *et al*. 2000; Reed 2009).

To set the baseline for such a PVA, genetic methods can be used to assess the natural amount of dispersal between areas. The margins of areas to be investigated in this way might be set for various reasons including habitat suitability, human alteration (eg the highway upgrade), or intrinsic differences such as genetic incompatibilities (the latter are unlikely in the case of koalas, Sherwin *et al.* 2000). In these reports, the AMRI and SCU are asked to assess dispersal between areas within Wardell KMP (deliverable 1), and between Wardell KMP and adjacent areas (deliverable 2).

The spatial arrangement of sampling within Wardell was evident in the SCU study, but not in the AMRI study – perhaps AMRI did not have access to detailed location data.

There are many different methods that use genetic data to estimate average levels of dispersal between areas for a PVA. The AMRI and SCU reports each use a number of different methods to assess genetic subdivision, most of which were not converted to dispersal estimates. In both studies, there was a general pattern of increasing genetic similarity at decreasing separation ("Isolation by Disatnce"), but there were no clear boundaries where one locality was genetically isolated from another.

In each report only one method specifically targeted dispersal, by identifying some individuals who might have dispersed in the most recent generation: koalas that were genetically assigned to a location other than the one in which they were sampled (AMRI); or koalas with first-order relatives in a location other than the one in which they were sampled (SCU).

Both studies indicated that dispersal was relatively high both between subpopulations within Wardell, and between Wardell and adjacent areas. As the authors point out, this dispersal probably does two things: opposes loss of genetic variation within subpopulations (thus potentially aiding future adaptability, Frankham *et al.* 2010), and forestalls immediate extinction, which is a risk in small isolated populations, including koalas (Lunney *et al.* 2002).

Therefore, a precautionary conservation management approach would be to avoid any reduction of the measured level of dispersal, unless there had been assessment of the potential effects of such reduction. However, if PVA-sensitivity analysis shows that a certain reduction of dispersal (x%) is not likely to significantly affect population viability, then it would be reasonable to implement particular management options, if it could be reliably demonstrated that these options would reduce dispersal by no more than x%.

SCU recommends that the genetic measures of dispersal be incorporated into the future PVA. There are two limitations to the use of AMRI's and SCU's genetic dispersal estimates as base-line dispersal for the PVA-sensitivity analysis. The first limitation is that sample sizes are unavoidably small, so that the estimates are subject to high error rates. This is compounded by the fact that these particular analyses only tell us about dispersal in the most recent generation. AMRI and SCU were correct to avoid methods that purport to assess dispersal over tens of generations, but have been criticised on various grounds.

However, there exists a Mutual Information method that can successfully assess dispersal over tens of generations, from genetic data (Sherwin 2006, 2010, Dewar et al 2011, Chao et al 2015). This method avoids the problems that beset other genetic diversity and dispersal measures, and, unlike the other methods, can deal with the widest possible range of population sizes (10 upwards) and dispersal rates (from one in a thousand, to one-third of the population, per generation; Sherwin 2006). All the data from AMRI and SCU should be analysed by the Mutual Information method, to produce robust multigeneration dispersal assessments; the Mutual Information calculations can be carried out in the freeware GENALEX (http://biology.anu.edu.au/GenAlEx) which was used for other tasks by AMRI. The GENALEX website also contains a guide for conversion of Mutual Information for microsatellites to a dispersal estimate. For the mitochondrial DNA, such a conversion could be achieved by following either Dewar (2011, equation 8) for each variable site, or Chao et al. (2015 supplement equations B5-7) for entire haplotypes.

These dispersal assessments from Mutual Information could then be used as baseline in the PVA-sensitivity analysis to investigate how the viability of the koala population might be affected if the upgrade of the Pacific Highway reduces dispersal below this baseline level. The PVA-sensitivity analysis should assess the effect of the highway upgrade, including any measures likely to increase or decrease the road corridor's permeability to koalas, such as fences or overpasses.

The other data that can be included in the PVA is AMRI's and SCU's estimates of genetic variation within subpopulations, which can be included in some PVA programs. Its inclusion will add to realism, especially allowing assessment of when the genetic diversity at Wardell might fall below the lowest levels listed in populations of koalas and other species, reviewed by AMRI.

Finally, in commissioning the analysis of sensitivity of population extinction to altered dispersal, I encourage the Roads and Maritime Services to require information on not only the most likely outcome, but also the worst-case outcome, to facilitate precautionary management.

DETAILED COMMENT – AMRI REPORT

SAMPLING THE LOCALITIES AND INDIVIDUALS:

From Wardell there were 38 samples for microsatellite and mitochondrial DNA. The lack of identification of position for East and West Wardell makes it difficult to assess their importance relative to the proposed highway upgrade, and to compare this to the SCU report, which uses other nomenclature. Also the tiny number of samples from East makes any conclusions weak.

Four other sites near the NSW/Queensland border (Macquarie, Coffs Harbour, Tyagarah and Coomera) were sampled for both microsatellite and mitochondrial DNA, plus a sample set from the whole koala range for mitochondrial DNA only.

THE GENES USED AND THEIR VALIDATION – DELIVERABLE 4.

1. Microsatellites – biparental inheritance

The analysis was based on a good number of genes -15, and appeared suitable for the tasks of determining variability within location, and differentiation and dispersal between locations. The genes were checked carefully. Two other microsatellite genes were excluded for good reasons, and 10% of individuals were independently re-genotyped. The probability of two individuals having the same microsatellite profile was low (10^{-15}), showing that a good battery of genes had been analysed. Variants at the 15 genes appeared to be inherited independently, so that each provided useful information for the analysis (no “linkage disequilibrium”). In most cases there was no evidence of non-random mating within site (ie, there were few cases of genes out of “HWE”).

2. Mitochondrial DNA – maternal inheritance

An 800 bp portion of the mitochondrial DNA control region was sequenced, and appeared suitable for the tasks of determining variability within location, and differentiation and dispersal between locations.

WITHIN-LOCALITY GENETIC VARIATION – DELIVERABLE 3.

Microsatellite diversity within locations was summarised by a suitable array of measures: allelic diversity, allelic richness, private alleles (AP on page 6, called Pa later in Table 1) expected and observed heterozygosity, Hardy-Weinberg equilibrium (HWE–Fis Table 1) and linkage disequilibrium. These measures were not out of the ordinary for koalas (Table 1).

For mitochondrial DNA, within-locality variation was assessed by suitable statistics - haplotypic diversity and nucleotide diversity - within Wardell and the four other main sites. Wardell values were not out of the usual for koalas, although 37 out of 38 individuals had the same mitochondrial genotype (haplotype) at Wardell.

CONNECTIVITY WITHIN WARDELL _DELIVERABLE 1.

And

CONNECTIVITY BEWTEEN WARDELL AND SURROUNDING AREAS – DELIVERABLE 2.

1. Microsatellites – biparental

Microsatellite geographic structure was assessed by a number of suitable methods: STRUCTURE, DAPC, F-statistics, AMOVA, Isolation-by-distance tests in Mantel, Spatial autocorrelation of pairwise relatedness in GENALEX 6.5. Many analyses were presented without saying which type of gene (microsatellite or mitochondrial) they were based upon; I believe that in all such cases, they were microsatellites.

The authors avoided specifying definitive management units, which I consider to be wise given the relatively low differentiation indicated by most measures. There was only one genetic cluster at Wardell (deliverable 1), and gradually increasing differentiation with distance from Wardell, but no sharp breaks, a pattern called “Isolation by Distance” (deliverable 2).

As well as the DAPC, there was also a PCA presented on P11 (Fig 3), but not described in the methods section. This appears to be an analysis of microsatellite data, though that is not stated. The PCA showed that the five koalas from "East of focal area" were scattered amongst those from the west of focal area. The text states that the data in Fig 3 come from within Wardell. Thus "East of focal area" appears to mean the East part of Wardell itself, rather than an area to the east of the Wardell area, which would be the interpretation in other parts of the document, where the whole of Wardell appears to be referred to as the "focal area". Perhaps for the purposes of the PCA, the "focal area" means the proposed upgraded highway. If that interpretation is correct, then there appears to be no justification, at least with this small sample, for considering the koalas on either side of the proposed upgraded highway to be members of distinct separate populations. This should be clarified with detailed geographic information for each individual, so that there could be direct comparison with the results of SCU, which is currently not possible.

There were only two exceptions to the pattern of low differentiation, but I would not prioritise these two findings over the general consensus that there is little geographic differentiation within Wardell or between Wardell and other populations). Fst and Phi-st did show significant departures from zero, but there are many criticisms of Fst, and only partial fixes for these criticisms (Sherwin 2010, Wang 2015). Phi-st likely suffers from many of the same problems as Fst, because Phi-st is also a variance partition and an "order 2" diversity measure (Hill, 1973), the two characteristics that are at the root of Fst's many problems.

Of course, low differentiation may be due to high dispersal, and some of these measures (including Fst) can be converted to measures of dispersal, but the authors wisely did not do so, given the criticisms mentioned already.

However, there was one assessment of dispersal in and out of Wardell by microsatellites. Microsatellite DNA is biparentally inherited, so it traces dispersal of both sexes. Microsatellites were used to assess dispersal by identifying some individuals who might have dispersed in the most recent generation: koalas that were genetically assigned to a location other than the one in which they were sampled. The assignment test used was in GENALEX 6.5. It showed that some individuals were likely to have moved between Wardell and nearby localities such as Coffs Harbour, but the authors noted that the conclusions were hampered by a lack of samples from localities immediately adjacent to Wardell. Only a small number of individuals were identified as potential dispersers, but it is worth noting that only small numbers are needed to halt genetic differentiation by chance processes in transmission (Kimura and Crow, 1970).

2. Mitochondrial DNA – female dispersal

Mitochondrial DNA generally confirmed the results of the microsatellite analysis, but indicated slightly reduced dispersal of females, relative to males

Mitochondrial DNA is maternally inherited, so traces female dispersal. Mitochondrial geographic structure was assessed by suitable methods: AMOVA/Phi-ST versus distance, and a haplotype network. The AMOVA showed that 92% of mitochondrial variation was within locations. This contrasts with 75% of biparentally-inherited microsatellite variation being within locations, suggesting limited female dispersal. However, Wardell mitochondrial haplotypes do occur elsewhere, so that there must be some female dispersal. Also, mitochondrial DNA suggested that one individual was an immigrant to the Wardell area.

DETAILED COMMENT – SCU REPORT

SAMPLING THE LOCALITIES AND INDIVIDUALS:

Tables 1 and 5 show 47 samples sourced from the Wardell KMP plus two adjacent localities to the north: Lynwood and Dalwood. This group of samples will be collectively referred to as "Wardell" throughout this assessment. There were also an additional 88 koalas outside Wardell, whose locality information was somewhat scattered in the document, but from Figure 4 it seems that there were three sample sets from localities immediately to the west of Wardell, named from north to south as 30 koalas from "North Lismore", 20 from "South Lismore" and 22 from "Western". There were also 16 other koalas from further to the west of Wardell ("Far-western").

THE GENES USED AND THEIR VALIDATION – DELIVERABLE 4 (not listed as such by SCU).

The SCU analysis used only microsatellite genes - fourteen of them, and adequate number. It is not clear if this set of genes overlaps the set of genes analysed by AMRI. The variation at these genes was sufficient to give a 99% chance that a non-parent would be excluded as a potential parent. Microsatellite analysis of one known parent-offspring pair gave a relatedness estimate of approximately 0.5 (the correct value for such a pair. These values give confidence to the subsequent work assigning first-order relatives (FOR – parent-offspring or full-sibling). It was stated that these microsatellites are "able to detect the presence of genetic differentiation amongst populations with a power of 0.975 or higher after 10 generations and assuming an effective population size of 50-200". It was not explained how this power analysis was carried out.

WITHIN-LOCALITY GENETIC VARIATION – DELIVERABLE 3 (not listed as such by SCU).

Microsatellite diversity within-locations was not unusual for koalas (Table 5). In the north part of Wardell, there was marginally higher genetic variation than in the south (Table 5, with no confidence limits, so the significance of the difference cannot be evaluated). In the north part of Wardell, there was also lower mean relatedness (Table 3). If real, these two differences could indicate that the north has larger population size, or that it receives more immigration from elsewhere.

CONNECTIVITY WITHIN WARDELL _DELIVERABLE 1.

Genetic Subdivision

There appears to be mild genetic substructure within the Wardell area, but no complete isolation.

On Page 10 it is stated that "Genetic neighbourhood size in the Wardell KMP was estimated to be 21-30 Km^2. This confirms a pattern of limited dispersal across the study area and the likely presence of multiple subpopulations." A genetic neighbourhood is the size of an area within which mating appears to be random. Fig 1 indicates that the Wardell area is about 6km x20km, so that multiple neighbourhoods a few km across could indeed fit into the Wardell area. However, note that neighbourhood calculations are based on the idea that the population is continuous over a much larger scale than the neighbourhood, so they give no indication of sharp boundaries – indeed they assume that no such boundaries exist.

Sharp boundaries were also not supported by the FOR analysis, which suggested that connectivity between localities within Wardell is greatest between the closest localities, and decreases with distance (called "Isolation by Distance" page 10, paragraph 2).

Fst and a related quantity Rst suggest subdivision within Wardell. These measures of genetic differentiation are relatively high between North and South Wardell, compared to their values for differentiation between Wardell and the two closest localities (South Lismore and Western) west. However, these values are presented with neither significance testing, nor confidence limits. I suspect that the latter would be so wide that the comparison is meaningless - Fst has poor statistical properties, as discussed above.

On page 12 it is stated that "We also reject a model in which the Wardell KMP is divided into an eastern and western subpopulation corresponding to the two large tracts of remnant schlerophyll woodland and forest". It should be clarified why this model was rejected, and where on the map are the two large remnant tracts. It is also not clear how to compare this result to AMRI's "East" and "West", but if the division is the same in the two reports, then AMRI's finding in their PCA would confirm the SCU assertion. However, the correspondence cannot be known until the geographic and genetic data for both studies are plotted on a single map.

Genetic Estimates of Dispersal

Only one method specifically targeted dispersal, by identifying some individuals who might have dispersed in the most recent generation: koalas with first-order relatives (FOR) in a location other than the one in which they were sampled. Fig 3 showed that first-order relative pairs were found to be shared between most parts of Wardell , though decreasingly so at greater distances. Most pertinently for the purpose of the study, on pages ii and 10 it is stated that the FOR analysis confirmed that dispersal occurs across the proposed highway upgrade, at two places: Bagotville in the south, as well as in the north.

CONNECTIVITY BEWTEEN WARDELL AND SURROUNDING AREAS – DELIVERABLE 2.

Similarly to the pattern within Wardell, there is some evidence that localities more distant to Wardell are more genetically differentiated from Wardell (Table 4, again without significance testing or confidence limits). This is also reflected in the pattern of inferred dispersal events (Fig 4).

The authors also suggest that dispersal into the northern Wardell area is indicated by its relatively high levels of genetic variation (Table 5), however, as discussed above, the difference is marginal and has no confidence limits to allow assessment of its significance.

It was asserted several times that dispersal was asymmetrical, but few data were available to confirm this. There are programs such as MIGRATE that can attempt to fit models of asymmetric dispersal to genetic data, but I suspect that these programs would fail to converge, due to lack of data. With the existing smaller dataset, it might be possible to infer directionality of dispersal from the FOR data, if (1) there are data on ages of members of each FOR pair, (2) it is assumed that the younger member of the pair is an offspring, and (3) it is assumed that offspring are more likely to disperse. These assumptions mean that such an analysis might have only dubious value.

OTHER

Page 17 talks of "the potential significance of the Southern subpopulation of the Wardell KMP as the remaining relatively pure gene pool for koalas in this region". It is not clear how genetic purity is defined, nor is it explained why genetic purity is needed. Generally, the opposite - higher genetic variability - is good for conservation management (Frankham et al 2010), unless there are problems of genetic incompatibility between different races of koalas, which no-one has every suggested, to my knowledge.

Page 17 says that the south part of Wardell is more like a "functional koala metapopulation" than the north. There are two definitions of "metapopulation" (Levins 1969, Hanski 1999), either of which could probably apply to both north and south Wardell. I recommend that this term should not be used without further explanation.

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27 September 2015

Assessment of Koala genetics reports for NSW Roads & Maritime services report.

The two reports, Neaves et al. and Norman et al. each report on genetic diversity and structure of koala populations within the Wardell koala metapopulation and in relation to other regional populations. Both generate and analyse microsatellite datasets for a set of samples provided by RMS, but that is about where the congruence across project ends.

I am satisfied by the technical quality of each analysis. Norman et al. use published microsatellite loci (which ones, ref?), whereas Neaves et al. appear to have generated new marker loci. Neaves et al. use technical replicates and explicitly mention use of positive and negative PCR controls to directly assess consistency and potential for cross-contamination. No mention of this is made in the Norman et al. report. That said, based on prior experience, I do trust the quality of data provided both groups.

The scale of sampling differs between the two groups. Neaves et al include just the 34 RMS samples from the Wardell KMP, whereas Norman et al. supplement these with other samples from this region. At broader scale, Neaves et al. compare Wardell with more geographically distant populations, whereas Norman et al. have finer-scale sampling across the NE NSW region. Based on the information provided by Norman et al., most samples provided are from north or west of the proposed highway upgrade – there are very few from east of the Highway. The corollary is that the power to directly model the potential impact of the road works is rather limited.

The analytical methods differ across studies, as do their conclusions. In all cases, the methods are applied appropriately. Both studies find relatively high genetic diversity in the Wardell KMP, but superficially they come to different findings about structuring and dispersal within the region. Neaves et al. focus on summary statistics (Fst; spatial autocorrelation), clustering (PCA, STRUCTURE) and assignment methods and conclude that there is little evidence for substructure within Wardell and at the larger regional scale (to SE Qld.). By contrast, Norman et al. focus on relatedness estimates, especially distances among inferred First-order relatives as a surrogate for dispersal. They conclude that there is local structuring, with a local genetic neighboohood size of ~ 30 km^2 but, paradoxically, also infer a high rate of dispersal to nearby regional populations around Lismore.

These marked differences reflect the different forms of analysis, as well as scales of sampling. The Neaves et al. approach will be strongly influenced by long-term average metapopulation dynamics, possibly including colonization and density changes accompanying anthropogenic changes to habitat structure across the region (as discussed in Norman et al.). By contrast, the focus on first-order relatives by Norman et al. is better suited to analyzing recent (1-2 generation) dispersal

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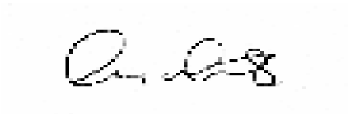
pattern, as is the finer-scale sampling across the region. Accordingly, I find the results of Norman et al. more relevant to the question at hand.

That said, I do have some reservations and recommendations:

1. The rather poor sampling east of the proposed new road limits the power to test directly for potential disruption of connectivity.
2. The results in Norman et al. are somewhat counter-intuitive in suggesting higher contemporary dispersal rates at large than small scale. Their analyses do not infer the direction of dispersal, so whether the southern populations are a source for those to the west remain speculation.
3. To address (1) in the context of the forthcoming PVA, it should be possible to model dispersal rate (using logistic regression on FOR distances or regression of pairwise r values) as a function of linear distance and habitat heterogeneity and with or without a potential road barrier
4. To address (2), methods that infer migration rates over recent generations (e.g. BayesAss) could used to test for asymmetry among north, south and adjacent regional populations.

Yours truly,



Appendix 3 – Australian Museum genetics report

Australian Centre for Wildlife Genomics
Australian Museum Research Institute

Koala population genetics management

A report to the Roads and Maritime Service (RMS)

12th August 2015

Linda E. Neaves, Siobhan B. Dennison,
Greta J. Frankham, Mark D. B. Eldridge and
Rebecca N. Johnson

nature culture **discover**

Australian museum

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Executive Summary

In April 2015, the Australian Centre for Wildlife Genomics (ACWG), Australian Museum Research Institute was engaged by the Roads and Maritime Service (RMS) to provide a genetic assessment of a Koala (*Phascolarctos cinereus*) population found within and around the Wardell area of the mid-north coast of New South Wales, with the aim of providing information on four project deliverables.

On the 17th June 2015, 38 Koala tissue samples were received by the ACWG at the Australian Museum in Sydney. Using standard operating procedures, DNA was successfully extracted from all 38 samples and a portion of the mitochondrial DNA (mtDNA) Control Region was sequenced. All 38 samples were successfully genotyped for 15 microsatellite loci (at the Australian Genome Research Facility, Melbourne).

Standard population genetics diversity indices for both the mtDNA and microsatellite data were generated for the Wardell Koala population and compared to data generated from four other sites in northern NSW and southern Queensland. The results of this analysis suggest that the levels of genetic diversity present in Wardell Koalas is comparable to that found at other locations in the region.

Analysis of the genetic structure among the Koalas sampled from Wardell revealed no pre-existing population sub-structure (Project deliverable 1). In a regional context there was evidence for gene flow across the populations sampled in the region (for both mtDNA and microsatellites), but with some genetic differentiation associated with geographic distance. This pattern indicates that populations that are geographically closer to one another are more closely related genetically than those that are geographically further apart. This is a pattern of differentiation often seen in wildlife population of species with some limitations to dispersal and therefore gene flow (Project deliverable 2)

Specific locus allele frequencies for the Wardell Koala population are given in Appendix 1 (Project deliverable 3)

Details of laboratory methods, data validation and analysis are given on pages 4-7 (Project deliverable 4).

Project Background

As part of its flagship research project, The Koala Genome Project, (http://koalagenome.org/drupal/) the Australian Centre for Wildlife Genomics (ACWG), at the Australian Museum Research Institute has an established research programme investigating the population genetics of northern New South Wales (NSW) Koala populations. The primary aim of this research is to understand the genetic diversity and structure of Koala populations in the Port Macquarie and Coffs Harbour regions. These populations have been examined to identify causes of population structuring, including natural biogeographic barriers or more recent barriers to gene flow such as major roads. This research project has recently expanded to also include populations from southern Queensland, Victoria as well as captive NSW Koalas, with the aim of collating one of the largest population genetics assessments of this species to date. Thus far over 250 individual Koalas have been sampled from northern NSW and south eastern Queensland (i.e. Port Macquarie, Coffs Harbour, Tyagarah, and Coomera) and analysed using both microsatellite markers and mitochondrial DNA (mtDNA).

Project Methods

Samples and DNA extraction:

A total of 38 tissues samples of Wardell Koalas were received by the ACWG on the 17th June 2015. Genomic DNA was extracted from the 38 tissue samples using the high salt method (Sunnucks and Hales, 1996) following standard protocols.

For comparison, additional samples held by the ACWG as part of an ongoing research programme investigating the population genetics of Koalas across their distribution (as outlined in Project Background), were also extracted using the protocol above and included in subsequent analyses. For microsatellite analyses, a total of 231 Koala samples from four surrounding locations – Port Macquarie, Coffs Harbour, Tyagarah and Coomera were genotyped and included as described in the Microsatellite section below. For mtDNA analyses a total of 454 Koala samples were included from across the species' distribution, including 243 from the four sites included in the microsatellite analyses as outlined in the Mitochondrial DNA section below.

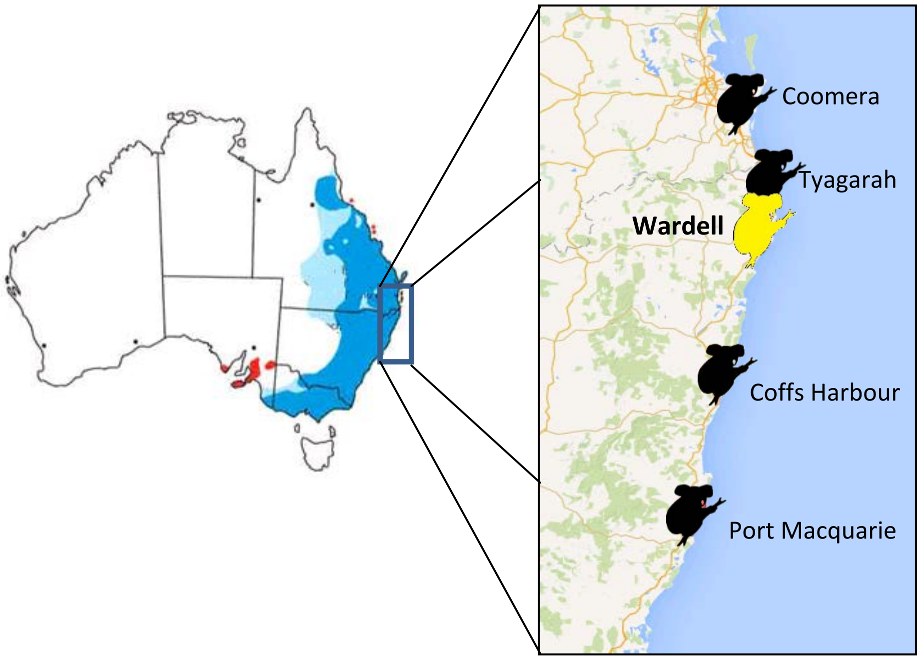

Figure 1: Map of Koala distribution, showing natural distribution (blue) and reintroduced sites (red). Insert shows the focal site (Wardell in yellow) and surrounding sampling locations in north-eastern NSW and south-eastern Qld (in black). Distribution map adapted from Van Dyck & Strahan (2008).

Microsatellite markers:

Development and amplification*:* To screen for tetra-, tri- and di-nucleotide microsatellite loci in the Koala, DNA was extracted from muscle tissue of one Koala specimen (Australian Museum Mammals #M.35147.004) using the DNeasy blood and tissue DNA extraction kit (QIAGEN). A total of 15.0µg of RNAse-treated genomic DNA was used in 1/8 of a plate for pyrosequencing by an external service provider, the Australian Genome Research Facility (www.agrf.com.au), on a Roche GL FLX (454) system. Using QDD (Meglécz et al. 2010), a program designed for both microsatellite detection and primer design for large data sets, we identified 1482 sequences that contained putative microsatellite motifs with five or more repeats and which had sufficiently-long flanking regions free of nanosatellites for which primers could be designed. 17 loci were identified that consistently and reliably amplified; these were optimised and used for genotyping.

Polymerase Chain Reaction (PCR) amplification was conducted using the following conditions: 94 °C for 5 min followed by 11 cycles at 94 °C (30 s), 60–55 ° (45 s; dropping 0.5 °C per cycle), and 72°C (45 s); followed by 19 cycles at 94°C (30 s), 55 °C (45 s), and 72 °C (45 s); followed by 8 cycles at 94 °C (30 s), 53 °C (45 s), and 72 °C (45 s); and a final extension step at 72 °C for 10 min. Multiplexed PCR products using labelled primers were run on a AB3730xl Sequencer by AGRF (Melbourne) and scored using GENEMAPPER 4.1 (Applied Biosystems). Any ambiguous genotypes were re-amplified. To ensure amplification and scoring consistency, at least 10% of samples at each locus were independently rerun and genotyped (Project deliverable 4). MICROCHECKER 2.2 (Van Oosterhout et al. 2004) was used to check for the presence of null alleles (Project deliverable 4).

Population genetic analyses:

Genetic diversity: To assess the levels of genetic diversity within the Wardell population and surrounding locations we calculated standard population genetics diversity indices, including allelic diversity (N_A), allelic richness (A_R; which allows for comparisons between sites comprising different sample sizes), private alleles ($A_{P;}$ the average number of alleles only found in that population) and levels of expected (H_E) and observed heterozygosity (H_O). Exact tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were conducted in GenAlEx 6.5 (Peakall & Smouse, 2012; 2006), GENEPOP 4.2 (Rousset 2008) and FSTAT 2.9 (Goudet 2001; Project deliverable 4).

Population genetic structure: The degree of genetic structure within the Wardell population (Project deliverable 1 & 3), and among surrounding Koala sampling localities (Project deliverable 2; i.e. Port Macquarie, Coffs Harbour, Coomera and Tyagarah – see Figure 1) was assessed using six different analytical approaches: STRUCTURE, DAPC, F-statistics, AMOVA, assignment tests, IBD (details provided below).

We calculated F_{ST} to assess levels of differentiation between sampling localities following the Weir & Cockerham (1984) approach in FSTAT 2.9.

An analysis of molecular variance (AMOVA) was implemented in GENALEX 6.5 to investigate if significant genetic partitioning occurred among our sampling locations, with significance calculated via bootstrapping (999 permutations).

To test for isolation by distance (IBD), a Mantel test was performed to test for a correlation between genetic and geographic distance matrices using genetic distances calculated in GENALEX 6.5.

To provide an indication of movement/gene flow between Wardell and surrounding sites, we undertook an assignment test in GENALEX 6.5. This test determines the likelihood of an individual originating from the population it was sampled in, based on allele frequencies within each population.

Spatial autocorrelation, implemented in GENALEX 6.5, was used to investigate spatial genetic structure within the sampled Wardell population. The SinglePops model was used to calculate average pairwise relatedness at various distance classes ranging from 50 m – 15 km, with significance values calculated by bootstrapping with 999 permutations.

To assess the levels of genetic structure both within the sampled Wardell population and surrounding populations within north-eastern NSW and south-eastern Qld, STRUCTURE 2.3 (Pritchard et al. 2000) was used to assess genotypic clustering and calculate assignment probabilities. Within Wardell, we examined values of K = 1-5, using the 'admixture' model with correlated allele frequencies, running ten replicates for each value of K, with 10^5 Markov Chain Monte Carlo (MCMC) iterations and 10^4 burn-in iterations. When examining all the sampled populations, analyses were run for values of K = 1-10 (twice the number of localities sampled). Two analyses were run using the 'admixture' model, one assuming correlated and one assuming independent allele frequencies. Again, ten replicates were run for each value of K, with 10^5 MCMC iterations and 10^4 burn-in iterations. The most likely number of genetic clusters (K) within Wardell and among sample localities was determined by first manually examining log-likelihood values and using the ΔK method of Evanno et al. (2005).

In addition to STRUCTURE 2.3, we used discriminant analysis of principal components (DAPC) to describe the genetic relationship among sampling localities. DAPC is a multivariate method that uses principal components analysis (PCA) to transform data into uncorrelated components, which are then analysed using a linear discriminant method (Jombart et al. 2010). This method is ideal for assessing small and potentially fragmented populations because, unlike STRUCTURE, it does not assume HWE and linkage equilibrium, which are assumptions often violated in natural populations. We used the R package, adegenet (Jombart 2008), implemented in R 2.12 (R development core team 2013; www.r-project.org) to run DAPC. Missing data were replaced with the mean, following Horne et al. (2011) and Dennison et al. (2015). The number of genetic clusters was selected using the find.clusters function and Bayesian Information Criterion (BIC). In DAPC, retaining too many principal components (PCs) as predictors with respect to sample size can result in over-fitting the model, while retaining too few can reduce statistical power. The a.score function in the R package, adegenet (Jombart 2008), suggests the optimal number of PCs to retain taking into account this trade off, so following a.score's recommendation, a conservative 8 PCs were retained for our DAPC analysis.

Mitochondrial DNA:

Amplification: A ~800 base pair (bp) fragment of Domain 1 of the mitochondrial DNA Control Region (*CR*) was also amplified from each individual using primers L15999M and H16498M (Fumagalli et al. 1997). PCRs were carried out in 25 µl reactions with 1000 ng of genomic DNA; BiolineMyTaq buffer; 2 pmol corresponding primers. Negative controls were included in each PCR. PCRs were performed on an Eppendorf Mastercycler EpS under the following conditions: initial denaturation (94 °C for 3 min); 36 cycles of denaturation (94 °C for 20 s); annealing (60 °C for 40 s) and extension (72 °C for 40 s) followed by a final extension (5 min at 72 °C). PCR products were cleaned using ExoSap-IT© (USB Corporation, Cleveland, Ohio, USA). Sequencing was resolved on an AB 3730xl Sequencer at AGRF Sydney, and verified. Any individuals with ambiguous sequence data or unique singleton haplotype (i.e. it was the only individual possessing that sequence) were re-amplified and verified. Five samples were re-amplified and sequenced as controls to ensure data integrity. Once verified, all sequences were aligned using the CLUSTAL X algorithm implemented in MEGA 6 (Tamura et al. 2013). Generated haplotypes were compared to existing published Koala haplotypes (available on Genbank; accession numbers AJ005846 to AJ005863; KJ530551 to KJ530556; KC505325; GQ851933 to GQ851940; AJ012057 to AJ012064; KF745869 to KF745875)

Data analysis: To estimate the levels of mtDNA *CR* diversity, haplotypic diversity (*h* – the number of different sequences/haplotypes present) and nucleotide diversity (π – the degree of differences between sequences; Tajima. 1983) were calculated within sites using ARLEQUIN 3.5.2.1 (Excoffier & Lischer 2010). These measures of genetic diversity were also calculated for surrounding localities (Port Macquarie, Coffs Harbour, Tyagarah and Coomera) for comparison.

The levels of differentiation at the mtDNA *CR* between Wardell and surrounding sampled populations were assessed via pairwise Φ_{ST}. Analysis of molecular variance (AMOVA), which utilises the distribution of, and sequence divergence between, haplotypes was implemented in ARLEQUIN 3.5.2.1 to assess how mtDNA diversity was partitioned (either within or between populations). We also assessed the influence of geographic distance by comparing the extent of differentiation (Φ_{ST}) with the geographic distance between populations. The significance was estimated using Mantel's test, with 10000 permutations in ARLEQUIN 3.5.2.1.

For the mtDNA *CR* haplotypes found in Wardell we constructed a haplotype network using the TCS procedure (Clement et al. 2002) implemented in PopART (http://popart.otago.ac.nz) to show how the different haplotypes were related. We then constructed a network containing all existing Koala mtDNA haplotypes to place Wardell in a broader distributional context.

Project Results

Genomic DNA was successfully extracted from all 38 samples provided from Wardell. All individuals were also successfully genotyped at 17 microsatellite loci and sequenced for a ~800 bp portion of the mtDNA *CR*. Two microsatellite loci were removed from subsequent analyses; locus Pcin01 was found to be monomorphic at all sampling localities and locus Pcin13 showed evidence of null alleles at Wardell and three out of the other four sampled populations. The probability of identity, which is the probability of obtaining two identical microsatellite genotype profiles by chance using this combination of loci was 1.7 x 10^{-15}, indicating that this is a powerful marker set. Comparisons of the duplicate individuals used to assess consistency of genotype scoring showed that all individuals were consistently scored across all loci. All of the remaining 15 microsatellite loci were found to be in HWE and there was no evidence for LD (Project deliverable 4). Two out of the five localities, Port Macquarie and Coffs Harbour were significantly out of HWE. This in not surprising, given sampling at these localities covered a broader geographical area than other sites and this disequilibrium is possibly due to a Wahlund effect from some within-site spatial structure (Wahlund 1928).

A summary and explanation of the results of each analysis undertaken to assess genetic diversity and structure (see Project deliverables 1 & 2) is provided below; separated into four sections; 1. Levels of genetic diversity, 2. Genetic sub-structuring within Wardell, 3. Gene flow between Wardell and surrounds, and 4. Genetic structure within north-eastern NSW/south-eastern Qld (i.e. Wardell in the context of the surrounding area).

1. Levels of genetic diversity

A summary of standard genetic diversity indices can be found in Table 1. Based on the samples provided, the Wardell population contained an average of 5 (±0.4) alleles per locus (range 3 – 9 alleles per locus), with an average expected heterozygosity of 0.62 (±0.03; Table 1). Two mtDNA *CR* haplotypes were identified, Pc7 and Pc13, which were separated by 11 base pairs and correspond to published haplotypes H5/Q1 and H2/Q8 respectively (Houlden et al. 1999; Fowler et al. 2000). These levels of diversity are similar to those found in other Koala populations in north-eastern NSW and southern Queensland (Table 1). However, compared with populations in the southern parts of the range, such as French Island and Western Victoria, Wardell exhibits high levels of genetic diversity (Houlden et al. 1999; ACWG unpublished data 2015). In general, while measures of genetic diversity obtained from different microsatellite loci are not directly comparable these indices of genetic diversity suggest that Koalas contain moderate levels of genetic diversity compared with other marsupials (Eldridge et al. 2010). For example, levels of genetic diversity appear lower in Koalas compared with highly mobile macropodids (e.g. western grey kangaroo; Neaves et al. 2009; 2012) and some arboreal species (e.g. ring-tailed possum, Lancaster et al. 2011) but are similar to those observed in other species with similar dispersal tendencies/habitat requirements, particularly in fragmented landscapes (e.g. long-nosed potoroo (Frankham et al. 2015) and spotted-tailed quoll (Firestone et al. 1999)).

Table 1: Summary of genetic diversity indices for Koala sampled from five sites in the north-eastern NSW and south-eastern Queensland

Locality	Microsatellites							Mitochondrial DNA			
	n	N_a ± SE	A_R ± SE	P_a ± SE	H_o ± SE	H_e ± SE	F_{IS}	*n*	H	*h* ± SD	π ± SD(%)
Wardell	**38**	**5.0 ± 0.4**	**3.8 ± 0.3**	**0.13 ± 0.09**	**0.61 ± 0.04**	**0.62 ± 0.03**	**0.025**	**38**	**2**	**0.06 ± 0.04**	**0.07 ± 0.06**
Port Macquarie	149	6.1 ± 0.5	4.1 ± 0.3	0.87 ±0.26	0.63 ± 0.03	0.65 ± 0.03	0.027	142	3	0.44 ± 0.03	0.10 ± 0.08
Coffs Harbour	57	6.1 ± 0.3	4.5 ± 0.3	0.47 ± 0.13	0.65 ± 0.03	0.67 ± 0.03	0.043	40	2	0.05 ± 0.04	0.02 ± 0.03
Tyagarah	17	4.3 ± 0.3	3.8 ± 0.3	0.27 ± 0.12	0.57 ± 0.06	0.59 ± 0.05	0.063	15	1	-	-
Coomera	8	3.3 ± 0.3	3.3 ± 0.3	0.13 ± 0.09	0.64 ± 0.07	0.56 ± 0.05	-0.082	8	1	-	-
Overall NE NSW/SE Qld	269	8.2 ± 0.6	5.0 ± 0.3	-	0.62 ± 0.02	0.62 ± 0.02	-	243	5	0.76 ± 0.02	0.66 ± 0.35

n = no. individuals sampled, N_a = no. alleles averaged over 15 loci, A_R = allelic richness averaged over 15 loci, P_a = private alleles averaged over 15 loci, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = inbreeding coefficient. H = number of haplotypes, *h*= haplotypic diversity, π = nucleotide diversity

2. Genetic sub-structuring within Wardell

We investigated patterns of genetic structure within the sampled Wardell population to determine if there was any sub-structuring present (Project deliverable 1). Multiple analyses (PCA, DAPC and STRUCTURE) all showed a single genetic unit within the focal area. That is, no genetic structure was detected. Spatial autocorrelation showed no significant genetic structure at all distance classes assessed within Wardell (Figure 2), and STRUCTURE and DAPC analyses (graphs not shown) assigned all individuals from Wardell to one genetic cluster. Furthermore, as Figure 3 shows individuals sampled from 'eastern' and 'western' regions of the focal area (based on data provided) form a single cluster. This lack of spatial genetic structure within the focal site is expected given the potential for Koalas to move large distances (Dique et al. 2003). Mitochondrial DNA also revealed no sub-structuring within Wardell, with 37 of the 38 individuals sampled sharing a haplotype.

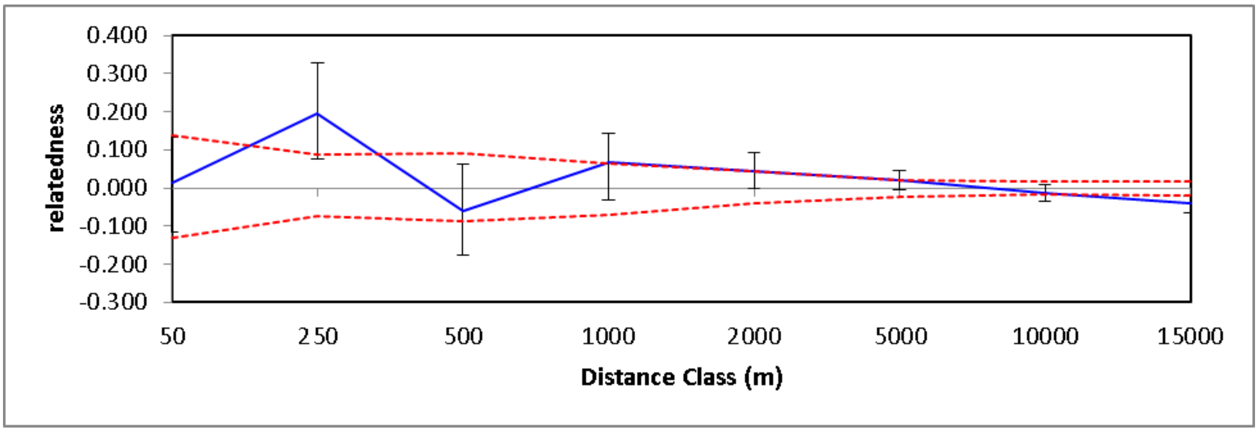

Figure 2. Spatial autocorellogram of Koalas sampled at Wardell. Average pairwise relatedness (± SE) is given at a range of distance classes. Red dotted lines represent the 95% confidence interval around which relatedness is effectively zero (i.e. random mating with no genetic structure).

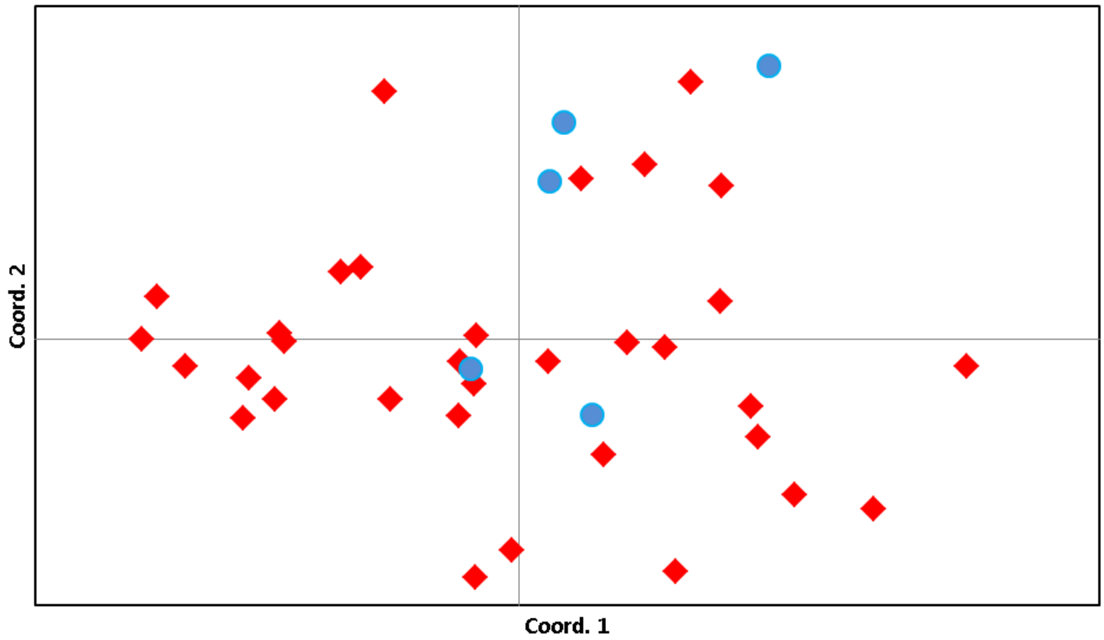

Figure 3: PCA plot showing the genetic distance between individuals within the Wardell focal area. Red diamonds represent individuals sampled in the west, while blue circles represent those sampled in the east of the focal area.

3. Gene flow between Wardell and surrounding locations

To determine how genetic diversity is partitioned among Wardell and surrounding sites we used an AMOVA. This analysis revealed that the genetic diversity present in the region is found primarily within sites, rather than partitioned between them (Table 2). This is reflected in the low numbers of private alleles (those found only within a single population) in Wardell or any other sampled population in the surrounding area (Table 1). A detailed summary of the by-locus allele frequency can be found in Appendix 1 (Project deliverable 3).

Mitochondrial DNA *CR* diversity showed a different pattern, reflecting the fact that mtDNA is maternally inherited. The AMOVA showed that mtDNA diversity was primarily partitioned between our sampled populations within the region surrounding Wardell, with only a small portion of the diversity found within populations. This result, likely reflects more limited movement in female Koalas, compared to males, leading to differences in the maternal lineages found at each site. On a larger landscape scale, however, the two haplotypes present at Wardell have also been recorded elsewhere in the range (see section 4 for details).

Table 2: AMOVA showing the partitioning of genetic diversity between and within Koala 'populations' for **a**. microsatellite markers and **b**. mitochondrial DNA

Source	df	SS	MS	Est. Var.	%
a. Microsatellite					
Among Pops	4	620.727	155.182	3.456	25%
Within Pops	264	2717.198	10.292	10.292	75%
Total	268	3337.926		13.748	100%
b. Mitochondrial DNA					
Among Pops	4	576.555		4.157	92.71
Within Pops	230	75.152		0.327	7.29
Total	234	651.707		4.484	100%

The levels of genetic differentiation varied between 0.101 and 0.217 among the sampled sites for microsatellites (F_{ST}) and between 0.000 and 0.991 for mtDNA data (Φ_{ST}) (Table 3). For the microsatellite data, there were significant levels of differentiation between all the sampled populations. Similarly, significant differentiation was found between all the sampled populations for mtDNA, with the exception of Tyagarah and Coomera. At this scale, these levels of population differentiation are expected given the geographic separation of our sampling sites and *do not* indicate an absence of gene flow between these locations. There was a significant positive correlation between genetic distance and the geographic distances separating sampling sites (Figure 4), an effect common in wildlife populations and known as isolation by distance. Hence, the further sampled sites were away from one another the more likely they were to be different, but each site is connected to others by gene flow from nearby locations. These data indicate gene flow occurs between Wardell and the surrounding areas.

Estimating numbers of animals moving between sites accurately is not possible without additional samples from nearby locations and, ideally, intensive tagging (either physical or genetic) and monitoring of individuals. However, to provide an idea of the movement of individuals between our sties we assessed the likelihood of individuals originating locally within Wardell. This analysis revealed evidence that at least two individuals appeared to originate from elsewhere (possibly the Coffs Harbour and Tyagarah areas), suggesting relatively recent immigration into the Wardell population. One of these individuals also possessed a distinct mtDNA haplotype to other Koalas in Wardell, further suggesting a non-local origin for this Koala. Additionally, examination of the other sampling localities suggested movement out of Wardell, with two individuals from

Coffs Harbour being more similar to Koalas found in Wardell than Coffs Harbour. Together these data indicate that gene flow has historically occurred amongst populations of Koalas throughout this region and maintenance of this gene flow (i.e maintenance of habitat corridors to facilitate this gene flow) is likely to be critical to maintaining genetic diversity within these populations in the future.

Table 3: Levels of differentiation between Wardell and surrounding locations. F_{ST} values calculated from microsatellite data are given above the diagonal and Φ_{ST} calculated from mitochondrial data are given below. * denotes significance at the 5% level.

	Wardell	**Port Macquarie**	**Coffs Harbour**	**Tyagarah**	**Coomera**
Wardell	-	0.180*	0.140*	0.115*	0.181*
Port Macquarie	0.933*	-	0.101*	0.217*	0.204*
Coffs Harbour	0.960*	0.204*	-	0.171*	0.155*
Tyagarah	0.961*	0.940*	0.991*	-	0.174*
Coomera	0.955*	0.937*	0.0990*	0.000	-

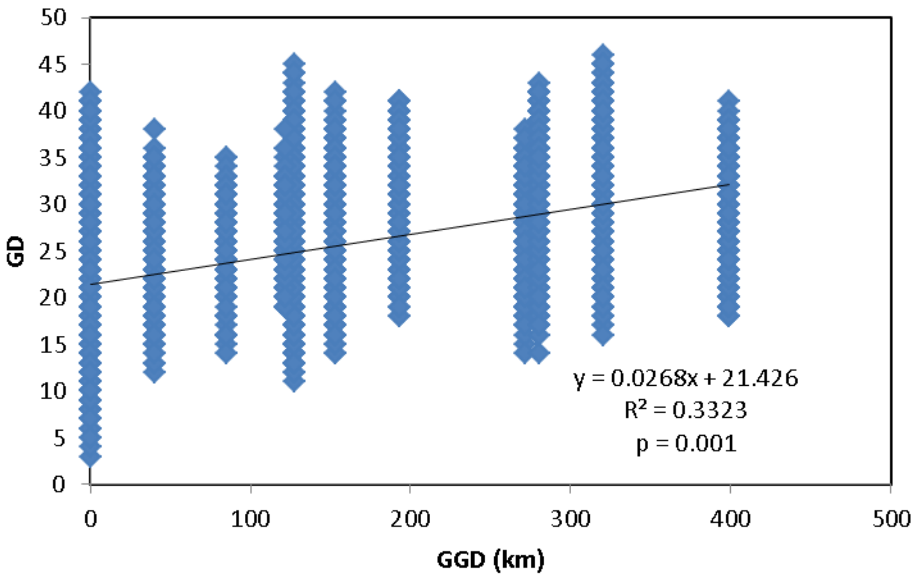

Figure 4: Relationship between genetic distance (GD; y-axis) and geographic distance (GGD; x-axis) between locations in north-eastern New South Wales and south-eastern Queensland based on microsatellite data. (Isolation by distance)

4. Genetic structure within north-eastern NSW and south-eastern Qld (i.e. Wardell in the context of surrounding locations

To place Wardell in a wider context we investigated the presence of genetic structure across the region for microsatellites, and across the distribution for mtDNA *CR* haplotypes. Figure 5 shows a PCA plot based on microsatellite genotypes, which provides a detailed picture of how genetically similar/dissimilar individual sampled Koalas are to one another. As the figure shows, the five sampled locations show some differences, reflecting the geographic distances that separate them, but there is no evidence of major genetic breaks within the region. This pattern is indicative of isolation by distance (as described above) and further sampling of intervening locations is likely to bridge the gaps between our localities.

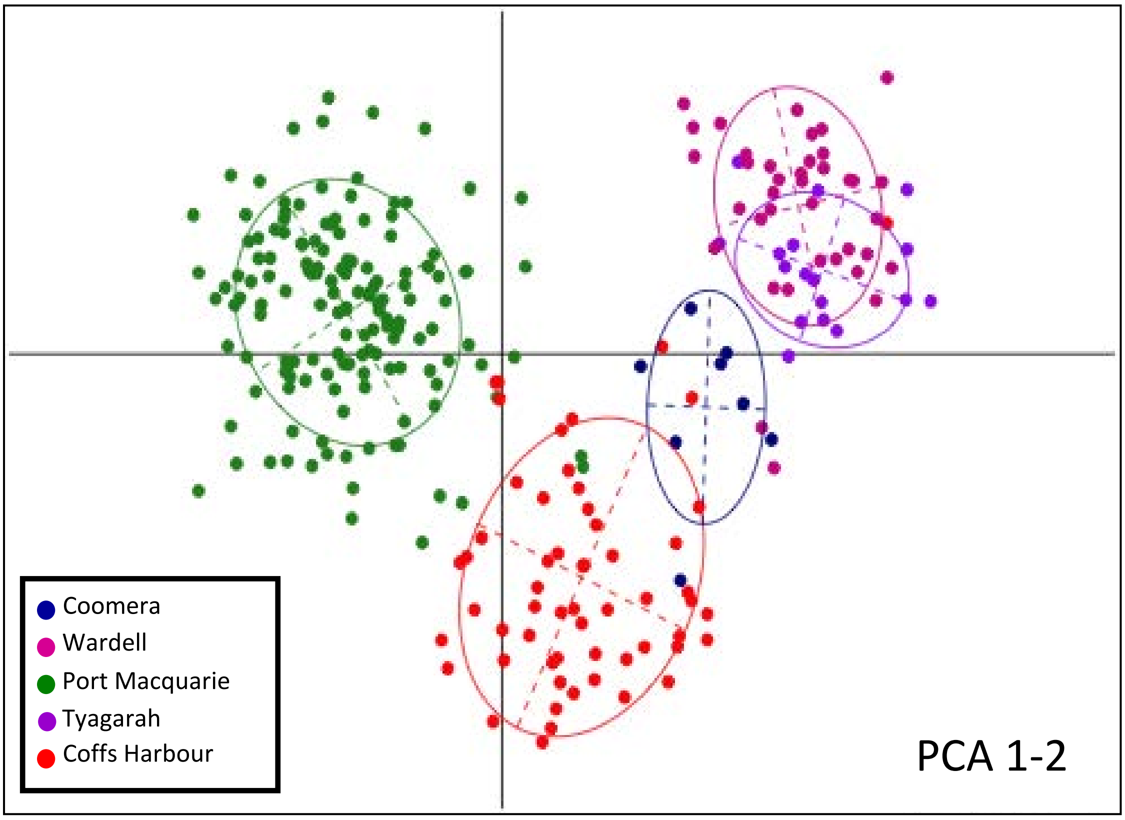

Figure 5: PCA plot showing Wardell and the four surrounding sampling sites. Each dot represents an individual Koala, and colours indicate the location from which they were sampled. The open circles represent the 95% confidence ellipses for each group.

STRUCTURE (using both correlated and independent allele frequencies) and DAPC analyses both indicated three genetic clusters are present in our dataset, which corresponded geographically to Port Macquarie, Coffs Harbour and the three northern-most sites – Wardell, Tyagarah and Coomera (Figure 6). Unlike the analyses undertaken to assess gene flow between sites (described in section 3), these methods are not reliant on sampling information and identify genetic clusters based on the genetic similarity between individuals (regardless of their geographic location). There is however, some overlap in the genetic clusters (DAPC scatterplot Figure 6a) suggesting gene flow occurs (or has occurred recently) between them and it is likely that additional sampling between the five sites will show further evidence of the gene flow between these clusters. These results highlight the importance of maintaining habitat connectivity between Koala populations to prevent isolation of any individual population or restricting gene flow along the coast, which may lead to the loss of genetic diversity and decline in population health.

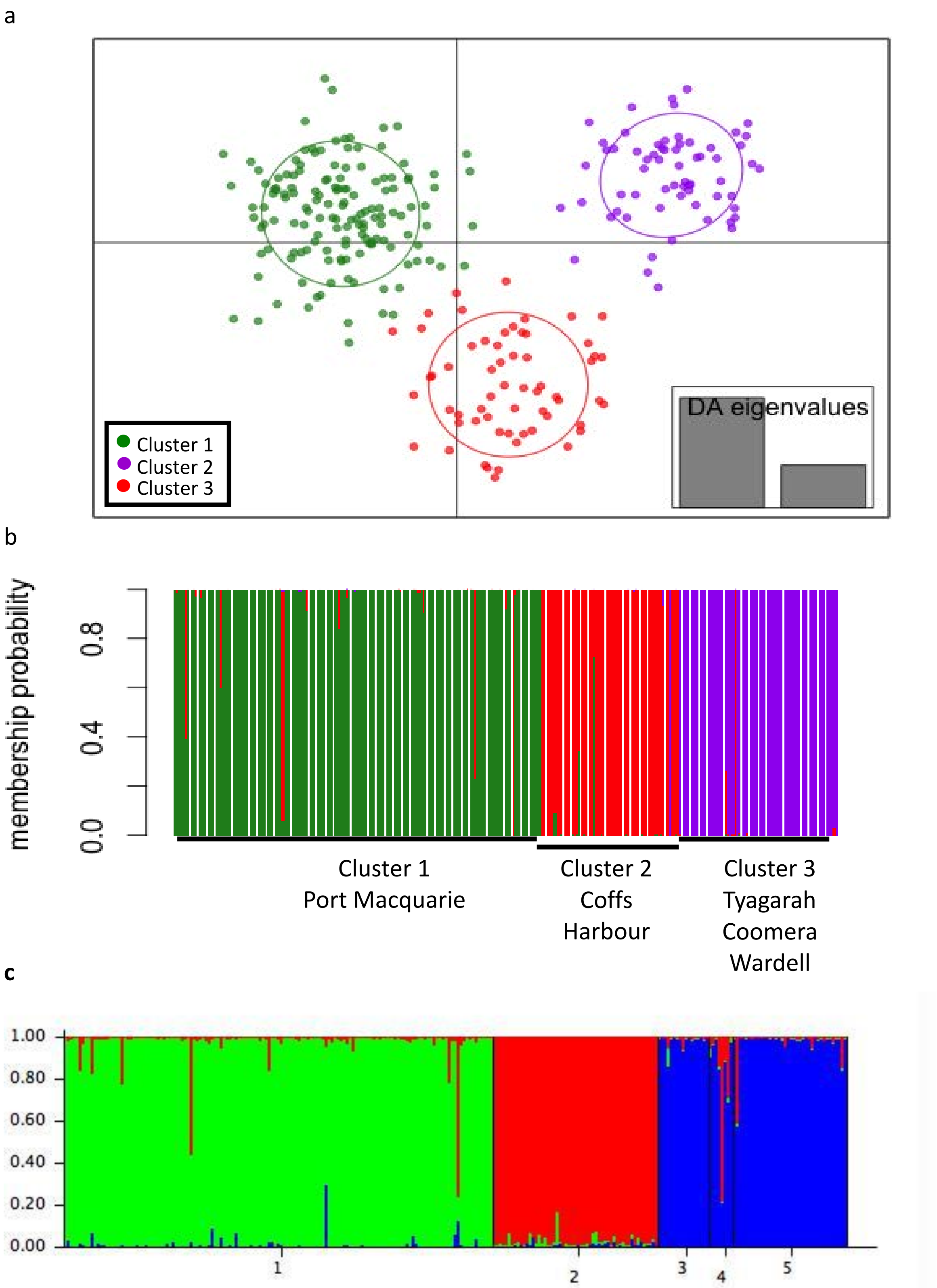

Figure 6. a. DAPC plot showing the three genetic clusters (encompassed by 95% confidence ellipses) identified in north-eastern NSW and south-eastern QLD. **b.** Plot showing the probability of assignment to the three potential genetic clusters generated by DAPC. Individuals are grouped according to the location they were sampled from. Cluster 1 corresponds to Port Macquarie, cluster 2 to Coffs Harbour and cluster 3 contains individuals from Tyagarah, Coomera and Wardell. **c.** Equivalent population assignment plot generated in STRUCTURE using the

“admixture” model with independent allele frequencies; 1 corresponds to Port Macquarie, 2 to Coffs Harbour, 3 to Tyagarah, 4 to Coomera and 5 to Wardell.

An analysis of mtDNA *CR* haplotypes from individuals distributed throughout the range, encompassing Qld, NSW, Vic and reintroduced Koala populations in South Australia (derived from samples held by ACWG and published data) revealed that overall there is little evidence of mtDNA genetic structure in Koalas. Within the north-eastern NSW/south-eastern Qld region, five haplotypes were identified, one (Pc7) was shared among Wardell, Tyagarah and Coomera, while Pc2 was shared between Coffs Harbor and Port Macquarie and the remaining three were unique to the populations they were sampled from (e.g. Pc13 in Wardell; Figure 7a). However, at the broader distributional scale for Koalas, very few haplotypes were restricted to a single location and several were present in multiple states (Figure 7b). For example, both haplotypes present in Wardell (Pc7 and Pc13) were also found elsewhere in NSW (e.g. Kyogle, Lismore, Narrandera, Tyagarah, Iluka Round Mountain, Tanglewood area) and Qld (e.g. Coomera, Gold coast and Mutdapilly; Figure 7b). Additionally, the haplotypes identified within the examined populations in north-eastern NSW and south-eastern Qld were present throughout the distribution (Figure 7a). The patterns observed in mtDNA data reflects a long history of gene flow across Koala populations but with some localized structure reflecting the more limited short-term dispersal of females.

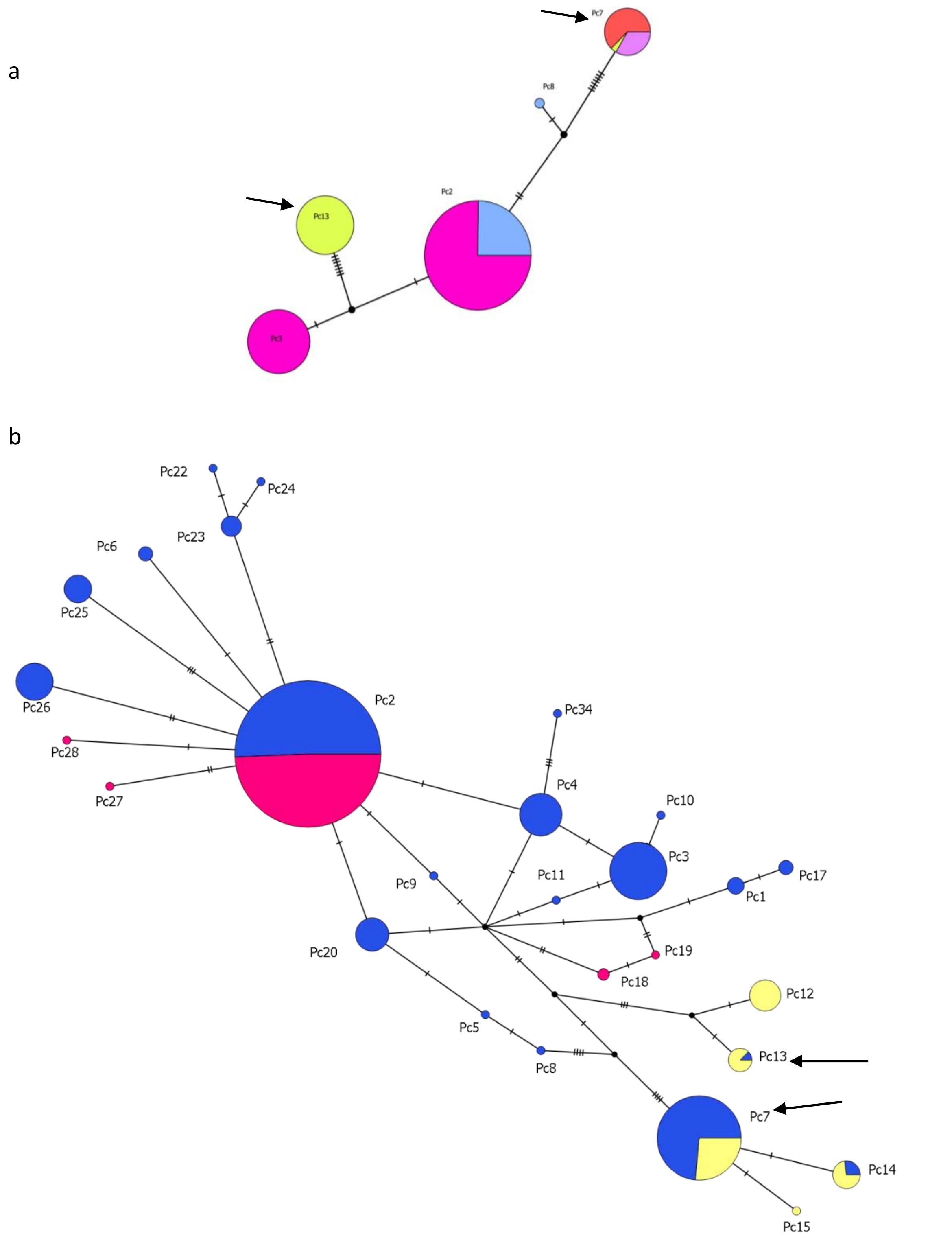

Figure 7: Mitochondrial DNA Control Region haplotype TCS networks for **a.** north-eastern NSW and south-eastern Queensland sites and **b.** all Koalas across the range. Hatches represent single base pair mutations, black circles are unsampled haplotypes and coloured circles are sampled haplotypes. The size of each circle reflects the number of times that haplotype was sampled and is divided according to the proportion of samples from each sampling location. **a.** Haplotypes from Wardell are shown in yellow, Tyagarah in red, Coomera in purple, Port Macquarie in pink and Coffs Harbour in blue **b.** Haplotypes from NSW are shown in blue, Qld in yellow and SA and Vic are shown in pink. Haplotype Pc7 and Pc13 were found in Wardell, indicated by the black arrows.

Summary

- Wardell is a genetically diverse Koala population, exhibiting similar levels of diversity to other sites sampled in this region (and higher than southern Koala populations)

- Based on the samples provided, there is no evidence of sub-structuring or spatial genetic structure within Wardell

- All sites sampled showed some degree of genetic differentiation consistent with isolation by distance.

- Gene flow is apparent between Wardell and other sites sampled in the surrounding area. In addition, there is evidence of recent movements of individuals, with two Wardell individuals appearing to have originated from outside Wardell.

- Genetically, Wardell is most similar to sampling locations further north (i.e. Tyaragah and Coomera) but there is evidence of a long history of gene flow (both nuclear and mitochondrial) throughout the region, and across the species' entire distribution.

Project Deliverables

The requested project deliverables are referred to throughout the report, but a summary is also provided below:

1. *Analyses of population structure and gene flow within the focal area will be conducted.* The results of these analyses revealed there was no evidence of genetic structure within the focal area. See section 2 for details.
2. *Analysis will be conducted to determine whether the focal population appears to be connected to populations in the surrounding area. For this analysis, animals from neighbouring regions closer to Ballina will be compared.* Gene flow connects the focal population to other sampled locations within the surrounding area (i.e. Port Macquarie, Coffs Harbour, Tyagarah and Coomera). See sections 3 and 4 for details
3. *By-locus allele frequency information for the population as a whole, and, if subpopulation structure is present, allele frequencies for each subpopulation will be reported.* A summary of the allele frequencies for each locus for all Wardell samples can be found at the end of this report. These values are for all individuals sampled at Wardell as there was no evidence of sub-structure.

4. *Summary of procedures undertaken for data validation will be reported (e.g. replicates and controls). Including testing for Hardy-Weinberg equilibrium and null alleles.* A summary of all the validation procedures can be found in the methods section of this report. In brief, this included the use of positive and negative controls in all laboratory protocols and at least 10% of all samples were replicated to ensure consistency in genotyping and sequencing. For microsatellite loci, tests for Hardy-Weinberg equilibrium, linkage disequilibrium and null alleles were conducted. The results of these analyses are described at the start of the results section.

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Appendix 1

Population allele frequencies for Koalas sampled in Wardell (n=38)

Locus	Alleles	Frequency
Pcin3		
	137	0.105
	141	0.013
	145	0.263
	149	0.039
	153	0.474
	157	0.105
Pcin5		
	176	0.342
	180	0.066
	188	0.276
	192	0.276
	200	0.039
Pcin6		
	153	0.447
	157	0.250
	161	0.303
Pcin7		
	133	0.434
	137	0.276
	141	0.158
	145	0.132
Pcin8		
	138	0.263
	142	0.566
	146	0.118
	158	0.026
	162	0.026
Pcin9		
	123	0.395
	125	0.250
	127	0.118
	129	0.013
	131	0.145
	135	0.079
Pcin10		
	129	0.197
	131	0.013
	141	0.763
	143	0.026

Pcin11		
	129	0.421
	133	0.053
	135	0.316
	139	0.013
	143	0.013
	145	0.066
	147	0.013
	149	0.105
Pcin14		
	132	0.224
	140	0.408
	142	0.013
	144	0.355
Pcin15		
	138	0.039
	140	0.105
	142	0.250
	144	0.105
	152	0.013
	154	0.158
	156	0.197
	158	0.118
	162	0.013
Pcin19		
	88	0.618
	97	0.289
	100	0.092
Pcin20		
	253	0.566
	256	0.421
	259	0.013
Pcin21		
	215	0.237
	221	0.079
	227	0.579
	230	0.092
	233	0.013
Pcin22		
	310	0.039
	313	0.013
	316	0.105
	319	0.066
	322	0.303

	325	0.474
Pcin23		
	87	0.026
	90	0.118
	96	0.776
	99	0.039
	102	0.039

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Australian Centre for Wildlife Genomics Results Report

Addendum Report

This is an addendum to the previous report by Neaves *et al.* (2015), relating to the genetic assessment of a Koala (*Phascolarctos cinereus*) population in the Wardell area of the mid-north coast of New South Wales, which was conducted by the Australian Centre for Wildlife Genomics (ACWG), Australian Museum Research Institute and submitted to the Roads and Maritime Service (RMS) on the 12th August 2015.

Addendum details:

On the 7th of October 2015 RMS requested further assistance in the interpretation of the results of genetic analyses, including undertaking two additional analyses:

(1) calculation of the genetic distance measure *Aij* (Rousett 2000) to estimate the size of the genetic neighbourhood, and;

(2) calculation of the relatedness coefficient (r) (Wang 2002);

These analyses were to be completed prior to the Koala Expert Advisory Committee workshop held on 14th October 2015.

Using the same 38 Koala samples provided by RMS from the Wardell area and 17 microsatellite markers described in the original report (Neaves *et al*. 2015), we calculated pairwise Wang's (2002) relatedness coefficient (hereafter, *r*) and Rousset's '*Aij'* (hereafter, *Aij*) using SPAGEDI v.1.5 (Hardy & Vekemans 2002).

The average *r* was -0.012 (SE = 0.008), the average *Aij* was 0.025 (SE = 0.005) and the average geographic distance between individuals was 6.5 km (SE = 0.16). No relationship between geographic distance and either of the genetic variables (*r* or *Aij*) was evident (Fig. 1), which is consistent with our initial findings (See Fig. 2 Neaves *et al.* 2015). A reliable estimate of genetic neighbourhood could not be calculated from the available data. This was most likely due to the limited spatial genetic structure detected (see below for an explanation).

The distances between potential first-order relatives (FOR = parent-offspring, full siblings) averaged 2.7 km, but ranged from 3 m to 11 km. This suggests substantial movement of

Koalas across the site and is consistent with the lack of genetic structure previously reported by Neaves *et al.* 2015 in Wardell.

These additional analyses are consistent with the patterns of dispersal previously reported in Neaves *et al.* (2015). The details of the analyses and results are described below and a complete list of the pairwise values is appended at the end of this report.

Yours sincerely,

Dr Rebecca Johnson, on behalf of the co-authors listed below.

Dr Linda Neaves, Siobhan Dennison, Dr Greta Frankham, and Dr Mark Eldridge

Accredited for compliance with (ISO/IEC 17025) interpreted for research using CITAC Guide CG2 "Quality Assurance for Research and Non Routine Analysis" (1998)

Details of Analyses and Results:

1. *Distance measure Aij (Rousett 2000) - estimate the size of the genetic neighbourhood*

Using the genotypic data generated from the initial analyses (Neaves *et al*. 2015), we calculated pairwise Rousset's '*Aij*' (hereafter, *Aij*) for the 38 Koala samples from Wardell provided by RMS using SPAGEDI v.1.5 (Hardy & Vekemans 2002). The average geographic distance between individuals was 6.5 km (SE = 0.16), and average *Aij* was 0.025 (SE = 0.005).

Genetic neighbourhood size (*Nb*) is calculated by regressing the slope of *Aij* and log-geographic distance over a restricted geographic range (Hardy & Vekemans 2002). We tested a range of geographic distance classes and densities based on those reported by Phillips and Chang (2013) and Phillips *et al*. (2015). However, the models failed to converge under all tested scenarios and therefore no reliable estimate of *Nb* (or gene dispersal; σ) could be calculated (Hardy *et al*. 2006). This can be attributed to (1) low marker polymorphism, (2) too narrow a sampling scale, or (3) weak spatial genetic structure (Hardy et al. 2006). Each of these limitation is likely to contribute to the lack of convergence in this instance, particularly point (3), as our previous analyses indicated Wardell Koalas exhibit little spatial genetic structure (Neaves *et al*. 2015). This is further demonstrated in the additional analyses reported here, as evidenced by the lack of a relationship between genetic and geographic distance (Fig. 1).

2. *Relatedness coefficient (R) (Wang 2002)*

Using the genotypic data generated from the initial analyses (Neaves *et al.* 2015), we calculated pairwise Wang's (2002) related coefficients (hereafter, *r*) for the 38 Koala samples from Wardell provided by RMS using SPAGEDI v.1.5 (Hardy & Vekemans 2002).

Relatedness values ranged from -0.59 (unrelated) to 0.69 (likely FOR), averaging -0.012 (SE = 0.008). As there were no data available on known relationship swe determined the range of potential relatedness values for FOR via a simulation analysis performed in CONANCESTRY (Wang, 2011; using the known allele frequencies for Wardell and simulating 1000 Koalas for 5 relatedness classes – parent-offspring, fullsibs, halfsibs, first cousins and unrelated). Based on this analysis we determined the relatedness values for FOR to be those greater than 0.38. This indicated 38 pairs (out of 703 pairwise comparisons) of Koala within Wardell were likely FOR. It should also be noted that based on the simulated dataset there is a 26% chance of misassignment of second-order relatives to FOR (and 10% for third-order relatives). The geographic distance between these likely FOR averaged 2.7 km and ranged from 3 m to 11 km. The proximity of some closely related Koalas may reflect daughters remaining near their mothers, as is observed in other marsupials (e.g. Frankham *et al.* 2012), but without associated data on the sex of Koalas this is uncertain. Regardless, these data indicate substantial movement of Koalas within Wardell consistent with previous finding of a lack of genetic structure (Neaves *et al*. 2015).

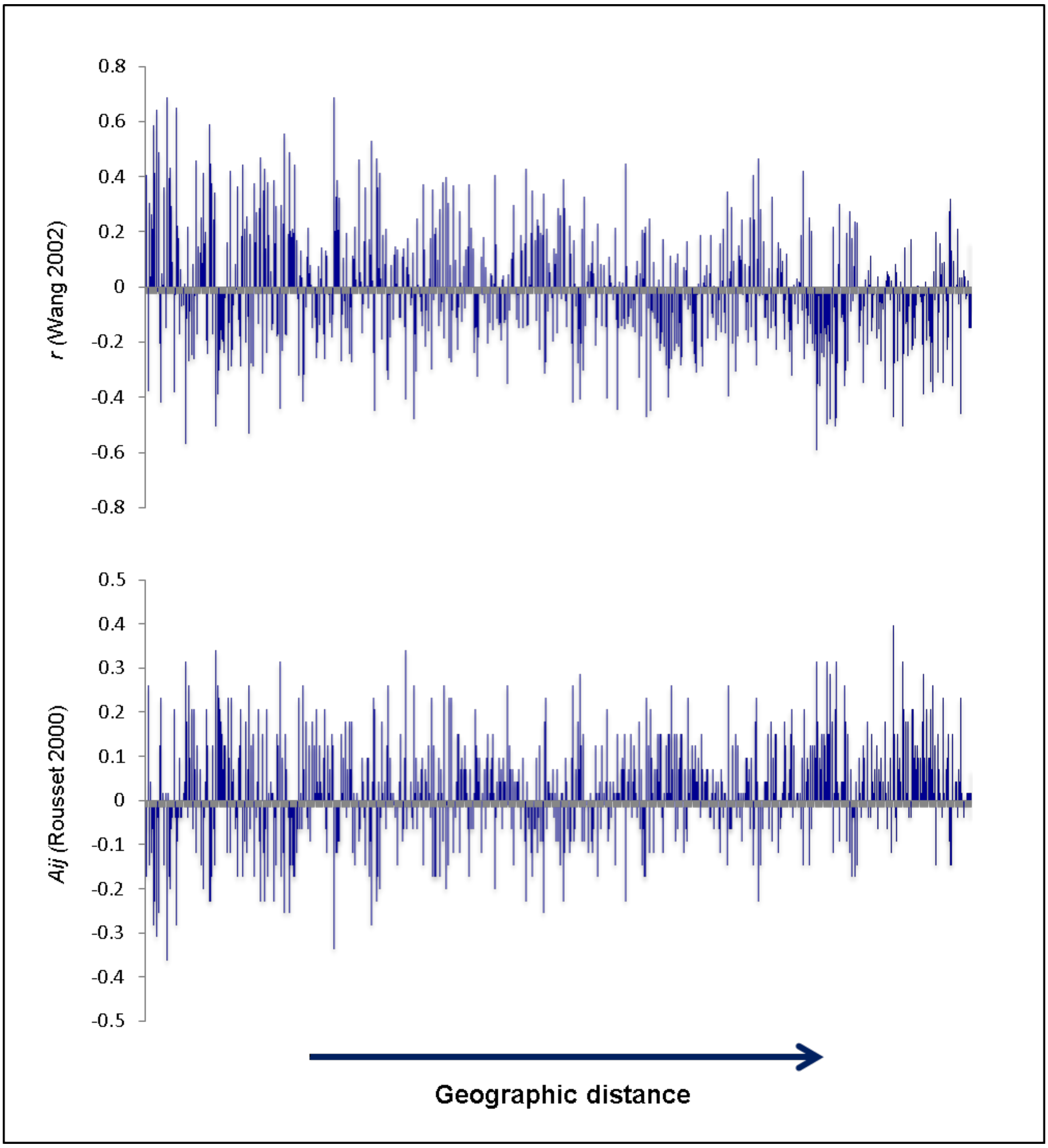

Fig. 1. The levels of genetic relatedness (top) and genetic distance (bottom) do not change substantially as geographic distance increases.

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Appendix

Table A1. Table of pairwise geographic distance (in km), relatedness (Wang 2002), and genetic distance (Rousset 2000) calculated from 38 Koala samples from Wardell.

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_001	AM161_002	0.237397	-0.381073	0.205714
AM161_001	AM161_003	12.926400	-0.390896	0.287143
AM161_001	AM161_004	1.861320	0.291838	-0.147143
AM161_001	AM161_005	0.646394	0.123779	0.070000
AM161_001	AM161_006	5.150700	-0.354003	0.260000
AM161_001	AM161_007	0.175913	0.291358	-0.065714
AM161_001	AM161_008	0.130168	0.360076	-0.147143
AM161_001	AM161_009	2.706850	0.105006	0.097143
AM161_001	AM161_010	1.555270	0.444454	-0.174286
AM161_001	AM161_011	3.191660	0.167136	-0.038571
AM161_001	AM161_012	1.537140	0.184017	-0.011429
AM161_001	AM161_013	3.230370	0.003577	0.097143
AM161_001	AM161_014	2.496780	-0.184191	0.151429
AM161_001	AM161_015	2.606190	-0.269831	0.151429
AM161_001	AM161_016	0.197806	0.090056	-0.038571
AM161_001	AM161_017	2.281970	-0.074766	0.178571
AM161_001	AM161_018	1.536220	-0.288812	0.205714
AM161_001	AM161_019	4.818350	-0.150762	0.205714
AM161_001	AM161_020	10.369100	-0.088946	0.097143
AM161_001	AM161_021	12.715400	-0.243904	0.205714
AM161_001	AM161_022	6.375840	0.107413	-0.065714
AM161_001	AM161_023	8.632660	-0.006720	0.015714
AM161_001	AM161_024	5.204250	-0.119914	0.042857
AM161_001	AM161_025	0.990095	-0.230178	0.205714
AM161_001	AM161_026	0.152577	0.394991	-0.174286
AM161_001	AM161_027	5.506360	-0.090293	0.042857
AM161_001	AM161_028	5.673190	-0.044258	0.015714
AM161_001	AM161_029	6.306640	0.003732	-0.011429
AM161_001	AM161_030	14.885400	0.022034	0.015714
AM161_001	AM161_031	8.121760	0.164990	-0.092857
AM161_001	AM161_032	0.155072	0.432837	-0.201429
AM161_001	AM161_033	12.465200	-0.001226	0.042857
AM161_001	AM161_034	12.532900	0.058614	-0.038571
AM161_001	AM161_035	12.520800	-0.210821	0.178571
AM161_001	AM161_036	12.520500	0.024965	-0.011429
AM161_001	AM161_037	12.153000	-0.137376	0.151429

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_001	AM161_038	11.101000	-0.206679	0.097143
AM161_002	AM161_003	12.712000	-0.504883	0.314286
AM161_002	AM161_004	1.933280	-0.233333	0.097143
AM161_002	AM161_005	0.784948	-0.244582	0.124286
AM161_002	AM161_006	5.085370	-0.193714	0.070000
AM161_002	AM161_007	0.105615	-0.420337	0.232857
AM161_002	AM161_008	0.122604	0.008976	0.015714
AM161_002	AM161_009	2.481030	-0.130472	0.070000
AM161_002	AM161_010	1.678970	-0.240822	0.205714
AM161_002	AM161_011	3.335850	-0.451579	0.205714
AM161_002	AM161_012	1.610290	-0.288506	0.151429
AM161_002	AM161_013	3.373430	-0.191304	0.178571
AM161_002	AM161_014	2.275880	-0.318379	0.070000
AM161_002	AM161_015	2.377360	-0.203407	0.042857
AM161_002	AM161_016	0.434100	-0.090117	0.097143
AM161_002	AM161_017	2.371020	-0.258360	0.205714
AM161_002	AM161_018	1.604470	-0.109267	0.070000
AM161_002	AM161_019	4.616770	-0.113879	0.042857
AM161_002	AM161_020	10.168600	-0.203877	0.042857
AM161_002	AM161_021	12.507700	-0.348821	0.124286
AM161_002	AM161_022	6.181630	-0.039225	-0.011429
AM161_002	AM161_023	8.515110	-0.288095	0.070000
AM161_002	AM161_024	5.045130	-0.114167	0.042857
AM161_002	AM161_025	1.105470	-0.200222	0.070000
AM161_002	AM161_026	0.089674	-0.205037	0.124286
AM161_002	AM161_027	5.341920	-0.139407	-0.038571
AM161_002	AM161_028	5.590990	-0.199520	0.070000
AM161_002	AM161_029	6.119810	-0.140639	-0.011429
AM161_002	AM161_030	14.702900	-0.463096	0.232857
AM161_002	AM161_031	7.920510	-0.400455	0.151429
AM161_002	AM161_032	0.138172	-0.149330	0.015714
AM161_002	AM161_033	12.235200	-0.481311	0.287143
AM161_002	AM161_034	12.303000	-0.276786	0.042857
AM161_002	AM161_035	12.291400	-0.475834	0.314286
AM161_002	AM161_036	12.290700	-0.506319	0.205714
AM161_002	AM161_037	11.943500	-0.361659	0.097143
AM161_002	AM161_038	10.881000	-0.237784	0.070000
AM161_003	AM161_004	12.703300	-0.269202	0.151429
AM161_003	AM161_005	12.969100	-0.180369	0.097143
AM161_003	AM161_006	10.185900	0.074332	0.070000

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_003	AM161_007	12.812800	-0.213436	0.205714
AM161_003	AM161_008	12.834200	-0.112622	0.097143
AM161_003	AM161_009	11.077800	-0.077185	0.042857
AM161_003	AM161_010	13.088000	0.057986	0.015714
AM161_003	AM161_011	13.869300	-0.347533	0.232857
AM161_003	AM161_012	12.711900	0.018631	0.042857
AM161_003	AM161_013	13.867700	0.086094	-0.038571
AM161_003	AM161_014	11.323000	-0.231957	0.124286
AM161_003	AM161_015	11.055100	-0.262528	0.205714
AM161_003	AM161_016	13.115700	-0.049553	0.124286
AM161_003	AM161_017	12.893100	0.004545	0.124286
AM161_003	AM161_018	12.678000	-0.473011	0.395714
AM161_003	AM161_019	8.149120	-0.083852	0.232857
AM161_003	AM161_020	2.903550	-0.244123	0.178571
AM161_003	AM161_021	0.816022	0.374444	-0.174286
AM161_003	AM161_022	6.713650	-0.154331	0.151429
AM161_003	AM161_023	7.585600	-0.129379	0.151429
AM161_003	AM161_024	8.318540	0.009804	0.097143
AM161_003	AM161_025	12.930400	-0.033764	0.097143
AM161_003	AM161_026	12.800200	-0.229608	0.205714
AM161_003	AM161_027	7.980910	-0.231424	0.151429
AM161_003	AM161_028	9.630090	0.148984	0.015714
AM161_003	AM161_029	6.893510	-0.047067	0.124286
AM161_003	AM161_030	3.956840	-0.141236	0.097143
AM161_003	AM161_031	4.938590	-0.003229	0.097143
AM161_003	AM161_032	12.846800	-0.186972	0.124286
AM161_003	AM161_033	2.468570	-0.005368	0.124286
AM161_003	AM161_034	2.446410	-0.263416	0.205714
AM161_003	AM161_035	2.336440	0.050970	0.124286
AM161_003	AM161_036	2.425330	0.030868	0.042857
AM161_003	AM161_037	0.959783	-0.304004	0.232857
AM161_003	AM161_038	1.958270	-0.171633	0.151429
AM161_004	AM161_005	1.241420	0.419715	-0.120000
AM161_004	AM161_006	3.692660	-0.147401	0.097143
AM161_004	AM161_007	1.975520	0.190830	-0.038571
AM161_004	AM161_008	1.936510	0.228325	-0.120000
AM161_004	AM161_009	4.004550	0.135324	-0.011429
AM161_004	AM161_010	0.504648	0.084208	-0.065714
AM161_004	AM161_011	1.612110	0.375924	-0.174286
AM161_004	AM161_012	0.324409	0.175828	-0.038571

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_004	AM161_013	1.639540	0.268806	-0.038571
AM161_004	AM161_014	3.870870	-0.306610	0.097143
AM161_004	AM161_015	3.866390	-0.172969	0.015714
AM161_004	AM161_016	1.851120	0.388085	-0.228571
AM161_004	AM161_017	0.458860	-0.247513	0.205714
AM161_004	AM161_018	0.328876	-0.172602	0.097143
AM161_004	AM161_019	4.632130	-0.076497	0.151429
AM161_004	AM161_020	9.958690	0.244259	-0.092857
AM161_004	AM161_021	12.380400	-0.303768	0.178571
AM161_004	AM161_022	5.990310	-0.154050	0.070000
AM161_004	AM161_023	7.422360	0.092206	-0.120000
AM161_004	AM161_024	4.480130	0.397074	-0.201429
AM161_004	AM161_025	0.897089	-0.173790	0.124286
AM161_004	AM161_026	1.925980	0.295415	-0.174286
AM161_004	AM161_027	4.817430	-0.241748	0.015714
AM161_004	AM161_028	4.298120	0.282796	-0.174286
AM161_004	AM161_029	5.829090	-0.019651	-0.065714
AM161_004	AM161_030	14.192000	0.094931	-0.011429
AM161_004	AM161_031	7.771090	-0.164700	-0.038571
AM161_004	AM161_032	1.978960	0.489574	-0.255714
AM161_004	AM161_033	12.602100	-0.155295	0.070000
AM161_004	AM161_034	12.666600	0.024184	-0.120000
AM161_004	AM161_035	12.638300	-0.370298	0.178571
AM161_004	AM161_036	12.651100	0.057801	-0.065714
AM161_004	AM161_037	11.854200	-0.351791	0.178571
AM161_004	AM161_038	11.010100	0.014284	0.015714
AM161_005	AM161_006	4.714100	-0.127354	0.097143
AM161_005	AM161_007	0.792157	0.446450	-0.228571
AM161_005	AM161_008	0.746916	0.087625	-0.011429
AM161_005	AM161_009	3.194890	0.362380	-0.147143
AM161_005	AM161_010	0.908882	0.245490	-0.065714
AM161_005	AM161_011	2.553490	0.688620	-0.337143
AM161_005	AM161_012	0.922235	0.343835	-0.147143
AM161_005	AM161_013	2.591520	0.321637	-0.092857
AM161_005	AM161_014	3.008480	0.111752	-0.120000
AM161_005	AM161_015	3.078430	0.027448	-0.011429
AM161_005	AM161_016	0.610070	0.147572	-0.011429
AM161_005	AM161_017	1.644900	-0.025274	0.042857
AM161_005	AM161_018	0.927262	-0.506780	0.341429
AM161_005	AM161_019	4.821380	-0.147947	0.124286

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_005	AM161_020	10.339000	0.325711	-0.092857
AM161_005	AM161_021	12.718600	-0.126956	0.015714
AM161_005	AM161_022	6.330370	-0.405063	0.205714
AM161_005	AM161_023	8.307440	-0.277627	0.124286
AM161_005	AM161_024	5.026860	0.154241	-0.038571
AM161_005	AM161_025	0.348953	-0.568531	0.314286
AM161_005	AM161_026	0.746955	0.414911	-0.201429
AM161_005	AM161_027	5.345340	0.036613	-0.065714
AM161_005	AM161_028	5.271180	0.131530	-0.011429
AM161_005	AM161_029	6.228450	0.049552	-0.065714
AM161_005	AM161_030	14.765900	0.033308	0.015714
AM161_005	AM161_031	8.101960	0.060382	-0.065714
AM161_005	AM161_032	0.785811	0.589788	-0.228571
AM161_005	AM161_033	12.629800	-0.082797	0.042857
AM161_005	AM161_034	12.696500	0.054111	-0.092857
AM161_005	AM161_035	12.678900	-0.275841	0.151429
AM161_005	AM161_036	12.683100	0.083688	-0.038571
AM161_005	AM161_037	12.167400	-0.197607	0.151429
AM161_005	AM161_038	11.184200	0.253474	-0.147143
AM161_006	AM161_007	5.175110	0.122635	0.070000
AM161_006	AM161_008	5.157390	-0.017600	0.070000
AM161_006	AM161_009	5.815390	-0.050998	0.097143
AM161_006	AM161_010	4.197290	0.097788	0.015714
AM161_006	AM161_011	4.113140	-0.046609	0.124286
AM161_006	AM161_012	3.914610	0.007499	0.042857
AM161_006	AM161_013	4.095500	-0.300310	0.232857
AM161_006	AM161_014	5.848460	0.221504	-0.120000
AM161_006	AM161_015	5.669360	0.283920	-0.092857
AM161_006	AM161_016	5.238720	-0.015335	-0.011429
AM161_006	AM161_017	3.584790	0.011623	-0.011429
AM161_006	AM161_018	3.892520	0.246722	-0.065714
AM161_006	AM161_019	3.555510	0.079763	0.015714
AM161_006	AM161_020	7.288470	0.246391	-0.120000
AM161_006	AM161_021	9.697210	0.114791	-0.038571
AM161_006	AM161_022	3.950980	-0.090568	0.070000
AM161_006	AM161_023	3.800730	-0.068402	0.042857
AM161_006	AM161_024	2.313940	0.108608	-0.038571
AM161_006	AM161_025	4.444320	0.379409	-0.092857
AM161_006	AM161_026	5.130430	0.043442	0.097143
AM161_006	AM161_027	2.555820	0.201345	-0.011429

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_006	AM161_028	0.652245	0.252932	-0.147143
AM161_006	AM161_029	3.646730	0.108463	-0.092857
AM161_006	AM161_030	10.953000	-0.158865	0.151429
AM161_006	AM161_031	5.431980	-0.124185	0.097143
AM161_006	AM161_032	5.196540	-0.024150	0.042857
AM161_006	AM161_033	10.716800	-0.049384	0.124286
AM161_006	AM161_034	10.771700	-0.127854	0.015714
AM161_006	AM161_035	10.715300	-0.190915	0.178571
AM161_006	AM161_036	10.751400	0.121719	-0.011429
AM161_006	AM161_037	9.253760	-0.307226	0.151429
AM161_006	AM161_038	8.815350	-0.163410	0.070000
AM161_007	AM161_008	0.047460	0.208937	-0.065714
AM161_007	AM161_009	2.532380	-0.101112	0.124286
AM161_007	AM161_010	1.697340	0.187610	0.015714
AM161_007	AM161_011	3.345140	0.415166	-0.201429
AM161_007	AM161_012	1.651360	0.470529	-0.228571
AM161_007	AM161_013	3.383350	0.081482	0.042857
AM161_007	AM161_014	2.321290	0.214134	-0.065714
AM161_007	AM161_015	2.432870	0.143337	0.015714
AM161_007	AM161_016	0.358886	-0.116428	0.178571
AM161_007	AM161_017	2.406550	0.077066	0.015714
AM161_007	AM161_018	1.647550	-0.135129	0.205714
AM161_007	AM161_019	4.720650	0.075082	0.070000
AM161_007	AM161_020	10.272400	0.100566	0.042857
AM161_007	AM161_021	12.610300	-0.057709	0.042857
AM161_007	AM161_022	6.286370	-0.143753	0.124286
AM161_007	AM161_023	8.615710	-0.111591	0.070000
AM161_007	AM161_024	5.150230	-0.120048	0.042857
AM161_007	AM161_025	1.126060	-0.039970	0.124286
AM161_007	AM161_026	0.049937	0.585616	-0.282857
AM161_007	AM161_027	5.447290	0.222436	-0.038571
AM161_007	AM161_028	5.684710	-0.011657	0.015714
AM161_007	AM161_029	6.225120	0.167086	-0.120000
AM161_007	AM161_030	14.808300	0.036784	-0.011429
AM161_007	AM161_031	8.024320	0.119925	-0.065714
AM161_007	AM161_032	0.034071	0.405756	-0.174286
AM161_007	AM161_033	12.328000	0.301132	-0.092857
AM161_007	AM161_034	12.395900	-0.050507	-0.038571
AM161_007	AM161_035	12.384700	-0.089622	0.070000
AM161_007	AM161_036	12.383700	0.275238	-0.120000

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_007	AM161_037	12.045600	-0.253631	0.151429
AM161_007	AM161_038	10.979700	-0.065062	-0.011429
AM161_008	AM161_009	2.576790	0.206376	-0.092857
AM161_008	AM161_010	1.653160	0.348292	-0.120000
AM161_008	AM161_011	3.299380	0.117012	-0.092857
AM161_008	AM161_012	1.612190	0.162545	-0.011429
AM161_008	AM161_013	3.337660	0.359330	-0.174286
AM161_008	AM161_014	2.366640	-0.113324	0.015714
AM161_008	AM161_015	2.476490	-0.030880	0.015714
AM161_008	AM161_016	0.318874	0.222415	-0.092857
AM161_008	AM161_017	2.365640	-0.014310	0.124286
AM161_008	AM161_018	1.608950	-0.278854	0.124286
AM161_008	AM161_019	4.736800	-0.077001	0.124286
AM161_008	AM161_020	10.288500	-0.033685	-0.011429
AM161_008	AM161_021	12.629000	-0.061137	-0.011429
AM161_008	AM161_022	6.300130	0.080746	0.015714
AM161_008	AM161_023	8.609000	-0.096115	0.015714
AM161_008	AM161_024	5.153530	0.043531	-0.011429
AM161_008	AM161_025	1.082610	-0.189427	0.151429
AM161_008	AM161_026	0.034535	0.305931	-0.147143
AM161_008	AM161_027	5.452060	-0.229818	0.124286
AM161_008	AM161_028	5.670330	0.261202	-0.147143
AM161_008	AM161_029	6.236500	-0.113799	0.015714
AM161_008	AM161_030	14.818600	-0.043451	-0.011429
AM161_008	AM161_031	8.040580	-0.285516	0.151429
AM161_008	AM161_032	0.042952	0.262577	-0.120000
AM161_008	AM161_033	12.357100	-0.101224	0.042857
AM161_008	AM161_034	12.424900	-0.143381	0.015714
AM161_008	AM161_035	12.413400	-0.027160	0.042857
AM161_008	AM161_036	12.412700	0.236443	-0.174286
AM161_008	AM161_037	12.065100	-0.150916	0.124286
AM161_008	AM161_038	11.003500	-0.135023	0.015714
AM161_009	AM161_010	3.961080	0.373802	-0.147143
AM161_009	AM161_011	5.594140	0.139803	-0.038571
AM161_009	AM161_012	3.737660	0.176948	-0.011429
AM161_009	AM161_013	5.625730	0.082687	0.015714
AM161_009	AM161_014	0.257676	-0.031388	-0.038571
AM161_009	AM161_015	0.151475	-0.026078	0.015714
AM161_009	AM161_016	2.886840	0.195307	-0.038571
AM161_009	AM161_017	4.458310	-0.187893	0.260000

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_009	AM161_018	3.715550	-0.410643	0.341429
AM161_009	AM161_019	3.631910	-0.113335	0.151429
AM161_009	AM161_020	8.840130	0.211345	-0.038571
AM161_009	AM161_021	11.004400	0.017454	-0.011429
AM161_009	AM161_022	5.154250	-0.085830	0.124286
AM161_009	AM161_023	8.436780	-0.217744	0.151429
AM161_009	AM161_024	4.701740	0.275589	-0.120000
AM161_009	AM161_025	3.432410	-0.339283	0.260000
AM161_009	AM161_026	2.556010	0.328571	-0.120000
AM161_009	AM161_027	4.883630	-0.029666	0.015714
AM161_009	AM161_028	6.103580	0.327387	-0.092857
AM161_009	AM161_029	5.226900	-0.149179	0.070000
AM161_009	AM161_030	13.584900	0.159551	0.015714
AM161_009	AM161_031	6.656140	-0.116504	0.070000
AM161_009	AM161_032	2.558500	0.388513	-0.120000
AM161_009	AM161_033	10.252400	-0.145517	0.097143
AM161_009	AM161_034	10.321800	-0.111559	0.042857
AM161_009	AM161_035	10.325100	-0.188791	0.151429
AM161_009	AM161_036	10.312500	-0.113786	0.042857
AM161_009	AM161_037	10.415000	-0.230475	0.151429
AM161_009	AM161_038	9.156390	0.094396	-0.011429
AM161_010	AM161_011	1.665560	0.138899	-0.065714
AM161_010	AM161_012	0.376092	0.218699	-0.038571
AM161_010	AM161_013	1.701620	0.057960	0.042857
AM161_010	AM161_014	3.800300	0.045711	-0.011429
AM161_010	AM161_015	3.832030	0.123652	-0.065714
AM161_010	AM161_016	1.500710	0.364850	-0.174286
AM161_010	AM161_017	0.777857	0.199307	0.042857
AM161_010	AM161_018	0.410033	-0.269924	0.260000
AM161_010	AM161_019	4.974850	0.043438	0.097143
AM161_010	AM161_020	10.370000	-0.013429	0.015714
AM161_010	AM161_021	12.783700	0.171356	-0.038571
AM161_010	AM161_022	6.382720	0.042942	0.042857
AM161_010	AM161_023	7.921810	-0.297938	0.151429
AM161_010	AM161_024	4.917410	0.070341	-0.065714
AM161_010	AM161_025	0.573541	-0.263712	0.205714
AM161_010	AM161_026	1.650610	0.286758	-0.092857
AM161_010	AM161_027	5.251930	0.186495	-0.065714
AM161_010	AM161_028	4.801680	0.137375	-0.065714
AM161_010	AM161_029	6.236350	-0.213403	0.124286

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_010	AM161_030	14.649600	0.035352	0.042857
AM161_010	AM161_031	8.166150	-0.021502	0.015714
AM161_010	AM161_032	1.693000	0.377728	-0.174286
AM161_010	AM161_033	12.917200	-0.208320	0.178571
AM161_010	AM161_034	12.982500	-0.204061	0.070000
AM161_010	AM161_035	12.957500	-0.193607	0.205714
AM161_010	AM161_036	12.967600	-0.122822	0.097143
AM161_010	AM161_037	12.250600	-0.243296	0.178571
AM161_010	AM161_038	11.367500	-0.173622	0.097143
AM161_011	AM161_012	1.856930	0.158250	-0.038571
AM161_011	AM161_013	0.042931	0.036597	0.042857
AM161_011	AM161_014	5.446330	0.243212	-0.120000
AM161_011	AM161_015	5.459610	0.195908	-0.092857
AM161_011	AM161_016	3.105180	-0.008078	0.015714
AM161_011	AM161_017	1.192700	-0.304366	0.232857
AM161_011	AM161_018	1.880010	-0.181660	0.151429
AM161_011	AM161_019	5.989020	-0.275660	0.178571
AM161_011	AM161_020	11.045300	0.421859	-0.147143
AM161_011	AM161_021	13.482100	-0.312331	0.151429
AM161_011	AM161_022	7.191430	-0.470855	0.232857
AM161_011	AM161_023	7.898090	-0.285030	0.124286
AM161_011	AM161_024	5.551060	0.052948	0.015714
AM161_011	AM161_025	2.235300	-0.322949	0.232857
AM161_011	AM161_026	3.300390	0.531339	-0.282857
AM161_011	AM161_027	5.889440	0.168064	-0.092857
AM161_011	AM161_028	4.765350	0.148451	-0.065714
AM161_011	AM161_029	6.983330	0.050341	-0.065714
AM161_011	AM161_030	15.020600	0.166032	-0.038571
AM161_011	AM161_031	8.938160	0.031529	-0.065714
AM161_011	AM161_032	3.337210	0.466636	-0.228571
AM161_011	AM161_033	13.954200	0.092311	0.015714
AM161_011	AM161_034	14.016700	0.131106	-0.147143
AM161_011	AM161_035	13.981300	-0.228015	0.097143
AM161_011	AM161_036	14.000000	0.273101	-0.092857
AM161_011	AM161_037	12.984000	-0.382875	0.260000
AM161_011	AM161_038	12.266400	0.216844	-0.120000
AM161_012	AM161_013	1.888150	-0.173789	0.124286
AM161_012	AM161_014	3.593890	0.135567	-0.147143
AM161_012	AM161_015	3.602730	-0.113198	0.042857
AM161_012	AM161_016	1.530580	-0.119402	0.097143

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_012	AM161_017	0.766276	0.158859	-0.038571
AM161_012	AM161_018	0.034137	-0.377977	0.260000
AM161_012	AM161_019	4.600350	0.368402	-0.120000
AM161_012	AM161_020	9.995630	0.081645	0.015714
AM161_012	AM161_021	12.408400	0.077063	-0.038571
AM161_012	AM161_022	6.007000	0.211645	-0.092857
AM161_012	AM161_023	7.605540	-0.231641	0.151429
AM161_012	AM161_024	4.549540	0.080206	-0.011429
AM161_012	AM161_025	0.583554	-0.035184	0.070000
AM161_012	AM161_026	1.601870	0.256135	-0.120000
AM161_012	AM161_027	4.883060	0.110388	-0.120000
AM161_012	AM161_028	4.504570	0.304861	-0.147143
AM161_012	AM161_029	5.861890	0.120805	-0.092857
AM161_012	AM161_030	14.287400	0.208894	-0.038571
AM161_012	AM161_031	7.790470	0.171651	-0.120000
AM161_012	AM161_032	1.654620	0.429096	-0.228571
AM161_012	AM161_033	12.547600	0.113572	-0.038571
AM161_012	AM161_034	12.612700	-0.269867	0.097143
AM161_012	AM161_035	12.587100	-0.015864	0.070000
AM161_012	AM161_036	12.597800	0.037737	-0.065714
AM161_012	AM161_037	11.874900	-0.031904	-0.011429
AM161_012	AM161_038	10.992400	0.030986	0.015714
AM161_013	AM161_014	5.479010	-0.313587	0.178571
AM161_013	AM161_015	5.490840	-0.275397	0.232857
AM161_013	AM161_016	3.144870	0.461117	-0.092857
AM161_013	AM161_017	1.215650	-0.131239	0.097143
AM161_013	AM161_018	1.910820	-0.441919	0.314286
AM161_013	AM161_019	5.997280	-0.407241	0.287143
AM161_013	AM161_020	11.041400	-0.085883	0.097143
AM161_013	AM161_021	13.478300	0.198689	-0.147143
AM161_013	AM161_022	7.192900	-0.077540	0.124286
AM161_013	AM161_023	7.878010	-0.216816	0.015714
AM161_013	AM161_024	5.549230	0.088008	0.042857
AM161_013	AM161_025	2.272100	-0.415771	0.260000
AM161_013	AM161_026	3.338470	0.067849	0.042857
AM161_013	AM161_027	5.887340	-0.419156	0.260000
AM161_013	AM161_028	4.747740	0.135720	0.070000
AM161_013	AM161_029	6.983230	-0.330843	0.178571
AM161_013	AM161_030	15.007900	-0.148505	0.015714
AM161_013	AM161_031	8.937670	-0.396378	0.260000

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_013	AM161_032	3.375610	0.186558	0.015714
AM161_013	AM161_033	13.960200	-0.051994	0.070000
AM161_013	AM161_034	14.022700	-0.360790	0.151429
AM161_013	AM161_035	13.986900	-0.183380	0.151429
AM161_013	AM161_036	14.005800	0.319123	-0.147143
AM161_013	AM161_037	12.981300	-0.343281	0.205714
AM161_013	AM161_038	12.268600	-0.074580	0.015714
AM161_014	AM161_015	0.277524	0.649825	-0.282857
AM161_014	AM161_016	2.672050	-0.100154	0.015714
AM161_014	AM161_017	4.327620	-0.055672	0.015714
AM161_014	AM161_018	3.573340	0.116777	-0.011429
AM161_014	AM161_019	3.808080	-0.040080	-0.038571
AM161_014	AM161_020	9.066780	0.056663	-0.065714
AM161_014	AM161_021	11.243200	0.203289	-0.065714
AM161_014	AM161_022	5.347920	0.009265	-0.011429
AM161_014	AM161_023	8.563790	0.001846	0.042857
AM161_014	AM161_024	4.829940	-0.326257	0.097143
AM161_014	AM161_025	3.260280	-0.078719	0.097143
AM161_014	AM161_026	2.347570	0.007811	-0.011429
AM161_014	AM161_027	5.024630	0.405210	-0.201429
AM161_014	AM161_028	6.161190	0.079985	-0.120000
AM161_014	AM161_029	5.409740	-0.000776	-0.065714
AM161_014	AM161_030	13.803100	0.043571	-0.011429
AM161_014	AM161_031	6.874180	-0.005764	-0.038571
AM161_014	AM161_032	2.346050	0.080183	-0.092857
AM161_014	AM161_033	10.508900	0.067678	-0.011429
AM161_014	AM161_034	10.578300	0.051256	-0.038571
AM161_014	AM161_035	10.581300	0.095928	0.015714
AM161_014	AM161_036	10.568900	0.140460	-0.065714
AM161_014	AM161_037	10.654700	-0.098125	0.042857
AM161_014	AM161_038	9.404450	-0.000512	-0.038571
AM161_015	AM161_016	2.789440	-0.052984	-0.011429
AM161_015	AM161_017	4.319110	-0.089750	0.070000
AM161_015	AM161_018	3.580130	0.147927	-0.038571
AM161_015	AM161_019	3.538010	-0.185030	0.097143
AM161_015	AM161_020	8.790180	0.157955	-0.120000
AM161_015	AM161_021	10.970800	0.005978	0.124286
AM161_015	AM161_022	5.073060	-0.138064	0.097143
AM161_015	AM161_023	8.317700	-0.028822	0.042857
AM161_015	AM161_024	4.582600	-0.085532	-0.011429

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_015	AM161_025	3.308610	-0.238378	0.232857
AM161_015	AM161_026	2.454480	0.132456	-0.065714
AM161_015	AM161_027	4.770350	0.370448	-0.174286
AM161_015	AM161_028	5.962240	0.012597	-0.065714
AM161_015	AM161_029	5.137800	-0.129343	0.042857
AM161_015	AM161_030	13.525600	-0.091996	0.070000
AM161_015	AM161_031	6.596700	-0.141476	0.070000
AM161_015	AM161_032	2.459980	0.113191	-0.120000
AM161_015	AM161_033	10.262900	-0.193399	0.178571
AM161_015	AM161_034	10.332100	0.066672	-0.065714
AM161_015	AM161_035	10.333800	-0.149155	0.178571
AM161_015	AM161_036	10.322500	-0.047354	0.042857
AM161_015	AM161_037	10.382800	-0.141567	0.151429
AM161_015	AM161_038	9.139600	-0.205603	0.124286
AM161_016	AM161_017	2.252260	0.130862	0.015714
AM161_016	AM161_018	1.534050	-0.180127	0.124286
AM161_016	AM161_019	5.001110	-0.158024	0.151429
AM161_016	AM161_020	10.550400	0.161585	-0.120000
AM161_016	AM161_021	12.900800	0.002231	0.097143
AM161_016	AM161_022	6.553760	0.018837	0.015714
AM161_016	AM161_023	8.757330	-0.047065	-0.065714
AM161_016	AM161_024	5.359970	0.349469	-0.174286
AM161_016	AM161_025	0.958012	-0.389599	0.260000
AM161_016	AM161_026	0.346615	0.013153	0.042857
AM161_016	AM161_027	5.665450	-0.167043	0.070000
AM161_016	AM161_028	5.773400	0.285269	-0.120000
AM161_016	AM161_029	6.479970	-0.446548	0.151429
AM161_016	AM161_030	15.054900	-0.085155	0.070000
AM161_016	AM161_031	8.303670	-0.245185	0.070000
AM161_016	AM161_032	0.330000	0.065511	-0.038571
AM161_016	AM161_033	12.661900	-0.050085	0.097143
AM161_016	AM161_034	12.729600	-0.071464	-0.038571
AM161_016	AM161_035	12.717300	-0.133556	0.178571
AM161_016	AM161_036	12.717200	0.142221	-0.011429
AM161_016	AM161_037	12.339500	-0.110559	0.042857
AM161_016	AM161_038	11.293300	-0.206500	0.124286
AM161_017	AM161_018	0.777945	-0.195782	0.205714
AM161_017	AM161_019	4.890150	0.182102	-0.011429
AM161_017	AM161_020	10.114900	-0.157804	0.097143
AM161_017	AM161_021	12.545200	0.006164	-0.011429

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_017	AM161_022	6.183160	0.057525	0.015714
AM161_017	AM161_023	7.363950	-0.084358	0.097143
AM161_017	AM161_024	4.612250	0.097485	-0.038571
AM161_017	AM161_025	1.295990	-0.288274	0.232857
AM161_017	AM161_026	2.357600	-0.149004	0.178571
AM161_017	AM161_027	4.952210	-0.206019	0.151429
AM161_017	AM161_028	4.213930	-0.144164	0.097143
AM161_017	AM161_029	6.001980	-0.206019	0.124286
AM161_017	AM161_030	14.261500	0.008716	0.042857
AM161_017	AM161_031	7.954700	-0.264113	0.260000
AM161_017	AM161_032	2.407490	-0.136878	0.124286
AM161_017	AM161_033	12.868700	-0.066755	0.070000
AM161_017	AM161_034	12.932400	0.018916	0.042857
AM161_017	AM161_035	12.900900	-0.039062	0.097143
AM161_017	AM161_036	12.916400	-0.054854	0.015714
AM161_017	AM161_037	12.029200	-0.168677	0.070000
AM161_017	AM161_038	11.234700	-0.133448	0.124286
AM161_018	AM161_019	4.566220	-0.272966	0.232857
AM161_018	AM161_020	9.962240	-0.028256	0.015714
AM161_018	AM161_021	12.374800	-0.360820	0.260000
AM161_018	AM161_022	5.973290	-0.072085	0.070000
AM161_018	AM161_023	7.579400	-0.186819	0.070000
AM161_018	AM161_024	4.517250	-0.259720	0.232857
AM161_018	AM161_025	0.592732	0.458700	-0.120000
AM161_018	AM161_026	1.597930	-0.200865	0.178571
AM161_018	AM161_027	4.850620	-0.005394	0.097143
AM161_018	AM161_028	4.480780	-0.027725	-0.011429
AM161_018	AM161_029	5.828490	-0.147874	0.042857
AM161_018	AM161_030	14.255800	-0.021785	0.042857
AM161_018	AM161_031	7.756770	-0.204480	0.151429
AM161_018	AM161_032	1.651510	-0.315749	0.151429
AM161_018	AM161_033	12.513600	-0.071361	0.097143
AM161_018	AM161_034	12.578700	-0.028244	0.015714
AM161_018	AM161_035	12.553100	-0.161452	0.151429
AM161_018	AM161_036	12.563700	-0.111588	0.042857
AM161_018	AM161_037	11.841100	-0.594565	0.314286
AM161_018	AM161_038	10.958300	-0.323017	0.205714
AM161_019	AM161_020	5.551790	-0.039716	0.015714
AM161_019	AM161_021	7.903900	-0.006639	0.015714
AM161_019	AM161_022	1.591030	0.211653	-0.092857

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_019	AM161_023	4.963030	0.047975	0.070000
AM161_019	AM161_024	1.429960	-0.067892	0.070000
AM161_019	AM161_025	4.783910	-0.016413	0.070000
AM161_019	AM161_026	4.702320	0.016543	0.070000
AM161_019	AM161_027	1.441800	0.081851	-0.065714
AM161_019	AM161_028	3.466800	-0.028299	-0.011429
AM161_019	AM161_029	1.606720	0.192788	-0.065714
AM161_019	AM161_030	10.141500	-0.056448	0.097143
AM161_019	AM161_031	3.303790	0.022120	0.042857
AM161_019	AM161_032	4.754260	-0.009071	0.015714
AM161_019	AM161_033	7.978620	-0.093928	0.070000
AM161_019	AM161_034	8.042350	-0.253415	0.151429
AM161_019	AM161_035	8.011370	-0.207369	0.151429
AM161_019	AM161_036	8.026340	-0.216613	0.070000
AM161_019	AM161_037	7.348150	-0.449712	0.205714
AM161_019	AM161_038	6.394110	-0.047284	0.042857
AM161_020	AM161_021	2.436920	-0.173652	0.097143
AM161_020	AM161_022	4.008760	-0.217218	0.070000
AM161_020	AM161_023	4.839800	-0.184045	0.042857
AM161_020	AM161_024	5.506140	0.210130	-0.065714
AM161_020	AM161_025	10.264800	-0.285524	0.232857
AM161_020	AM161_026	10.254000	0.405804	-0.147143
AM161_020	AM161_027	5.165970	0.101215	-0.065714
AM161_020	AM161_028	6.727400	0.445676	-0.228571
AM161_020	AM161_029	4.133760	0.213461	-0.174286
AM161_020	AM161_030	4.794590	0.215440	-0.065714
AM161_020	AM161_031	2.248330	0.040936	-0.065714
AM161_020	AM161_032	10.306100	0.282070	-0.147143
AM161_020	AM161_033	4.090120	0.174977	-0.038571
AM161_020	AM161_034	4.120390	0.192660	-0.174286
AM161_020	AM161_035	4.027740	-0.087172	0.042857
AM161_020	AM161_036	4.095580	0.354552	-0.174286
AM161_020	AM161_037	1.965340	-0.176971	0.070000
AM161_020	AM161_038	2.043040	0.178713	-0.038571
AM161_021	AM161_022	6.403990	0.001872	0.015714
AM161_021	AM161_023	6.917260	0.039959	0.015714
AM161_021	AM161_024	7.940240	0.113533	0.015714
AM161_021	AM161_025	12.659100	0.038593	-0.011429
AM161_021	AM161_026	12.594700	-0.185984	0.124286
AM161_021	AM161_027	7.600210	0.024843	0.042857

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_021	AM161_028	9.119810	0.290709	-0.065714
AM161_021	AM161_029	6.551280	-0.149794	0.015714
AM161_021	AM161_030	3.362640	0.030349	-0.038571
AM161_021	AM161_031	4.621700	-0.229496	0.151429
AM161_021	AM161_032	12.644200	0.022872	0.015714
AM161_021	AM161_033	3.184450	-0.062813	0.042857
AM161_021	AM161_034	3.171550	-0.170273	0.097143
AM161_021	AM161_035	3.059820	0.102314	0.015714
AM161_021	AM161_036	3.148780	0.054535	-0.065714
AM161_021	AM161_037	0.597150	-0.172780	0.124286
AM161_021	AM161_038	2.158620	-0.043488	-0.065714
AM161_022	AM161_023	3.939890	-0.037757	0.015714
AM161_022	AM161_024	1.712240	-0.156874	0.015714
AM161_022	AM161_025	6.258340	-0.006081	0.015714
AM161_022	AM161_026	6.265590	-0.016191	0.042857
AM161_022	AM161_027	1.411670	-0.230235	0.042857
AM161_022	AM161_028	3.598460	-0.021347	-0.011429
AM161_022	AM161_029	0.332182	-0.071004	-0.038571
AM161_022	AM161_030	8.551020	0.014655	-0.011429
AM161_022	AM161_031	1.783520	-0.136666	0.015714
AM161_022	AM161_032	6.319650	-0.146127	0.042857
AM161_022	AM161_033	6.849000	-0.110036	0.070000
AM161_022	AM161_034	6.907740	-0.163740	0.070000
AM161_022	AM161_035	6.861140	-0.078364	0.151429
AM161_022	AM161_036	6.889000	-0.145815	0.042857
AM161_022	AM161_037	5.867870	-0.205459	0.097143
AM161_022	AM161_038	5.077500	-0.131752	0.042857
AM161_023	AM161_024	3.735180	0.073387	-0.065714
AM161_023	AM161_025	8.084500	-0.049641	0.070000
AM161_023	AM161_026	8.577670	-0.186440	0.070000
AM161_023	AM161_027	3.570590	-0.118972	0.015714
AM161_023	AM161_028	3.155850	0.017533	-0.011429
AM161_023	AM161_029	3.672400	0.138089	-0.065714
AM161_023	AM161_030	7.409420	-0.093023	0.015714
AM161_023	AM161_031	4.089520	0.031783	0.015714
AM161_023	AM161_032	8.642840	-0.193383	0.042857
AM161_023	AM161_033	8.875790	-0.093142	0.015714
AM161_023	AM161_034	8.913110	0.345476	-0.147143
AM161_023	AM161_035	8.827720	0.022446	0.042857
AM161_023	AM161_036	8.888970	-0.166914	0.015714

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_023	AM161_037	6.630040	0.013914	0.097143
AM161_023	AM161_038	6.809030	-0.133824	0.042857
AM161_024	AM161_025	4.891010	-0.061595	0.070000
AM161_024	AM161_026	5.119830	0.025924	-0.065714
AM161_024	AM161_027	0.340205	-0.065595	0.015714
AM161_024	AM161_028	2.096820	0.444756	-0.174286
AM161_024	AM161_029	1.455020	-0.011299	-0.038571
AM161_024	AM161_030	9.749380	0.096135	0.015714
AM161_024	AM161_031	3.394940	-0.302975	0.124286
AM161_024	AM161_032	5.181050	0.295589	-0.147143
AM161_024	AM161_033	8.560520	0.042764	0.042857
AM161_024	AM161_034	8.618990	0.005456	-0.065714
AM161_024	AM161_035	8.571470	0.079975	0.070000
AM161_024	AM161_036	8.600110	0.189828	-0.038571
AM161_024	AM161_037	7.434780	-0.114747	0.070000
AM161_024	AM161_038	6.760610	0.075115	-0.011429
AM161_025	AM161_026	1.078740	-0.156134	0.178571
AM161_025	AM161_027	5.216400	-0.029662	0.042857
AM161_025	AM161_028	5.016880	0.013344	0.015714
AM161_025	AM161_029	6.139370	0.020636	-0.011429
AM161_025	AM161_030	14.640100	-0.063001	0.070000
AM161_025	AM161_031	8.037770	-0.130047	0.097143
AM161_025	AM161_032	1.123130	-0.241107	0.124286
AM161_025	AM161_033	12.657800	0.046140	0.042857
AM161_025	AM161_034	12.724100	-0.252423	0.178571
AM161_025	AM161_035	12.703400	-0.017357	0.070000
AM161_025	AM161_036	12.710000	-0.089163	0.097143
AM161_025	AM161_037	12.114500	-0.499129	0.314286
AM161_025	AM161_038	11.169600	-0.103629	0.070000
AM161_026	AM161_027	5.418150	0.170564	-0.065714
AM161_026	AM161_028	5.641830	0.122088	-0.092857
AM161_026	AM161_029	6.202000	0.087893	-0.092857
AM161_026	AM161_030	14.784200	0.062161	-0.038571
AM161_026	AM161_031	8.006100	0.086657	-0.092857
AM161_026	AM161_032	0.066233	0.413656	-0.228571
AM161_026	AM161_033	12.324900	0.082096	0.015714
AM161_026	AM161_034	12.392700	0.177557	-0.174286
AM161_026	AM161_035	12.381100	-0.270706	0.151429
AM161_026	AM161_036	12.380400	0.194222	-0.092857
AM161_026	AM161_037	12.030800	-0.238554	0.151429

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_026	AM161_038	10.969900	-0.004695	-0.038571
AM161_027	AM161_028	2.268520	0.032270	-0.065714
AM161_027	AM161_029	1.135190	0.163064	-0.120000
AM161_027	AM161_030	9.424090	-0.180358	0.124286
AM161_027	AM161_031	3.061740	0.218974	-0.147143
AM161_027	AM161_032	5.478560	0.339900	-0.255714
AM161_027	AM161_033	8.250010	-0.066536	0.070000
AM161_027	AM161_034	8.307840	-0.311901	0.042857
AM161_027	AM161_035	8.258710	-0.198044	0.124286
AM161_027	AM161_036	8.288700	-0.096046	0.070000
AM161_027	AM161_037	7.095530	-0.180945	0.070000
AM161_027	AM161_038	6.434230	-0.118975	0.015714
AM161_028	AM161_029	3.277650	0.172174	-0.174286
AM161_028	AM161_030	10.310000	0.167202	-0.011429
AM161_028	AM161_031	4.957350	-0.130977	0.015714
AM161_028	AM161_032	5.708060	0.390864	-0.228571
AM161_028	AM161_033	10.256200	0.242522	-0.065714
AM161_028	AM161_034	10.309100	-0.028167	-0.065714
AM161_028	AM161_035	10.248600	0.252856	-0.065714
AM161_028	AM161_036	10.288200	0.466672	-0.228571
AM161_028	AM161_037	8.690390	-0.139601	0.042857
AM161_028	AM161_038	8.318900	0.186620	-0.038571
AM161_029	AM161_030	8.585530	0.048112	-0.038571
AM161_029	AM161_031	1.955960	0.555773	-0.255714
AM161_029	AM161_032	6.257980	0.228841	-0.174286
AM161_029	AM161_033	7.115560	0.207209	-0.147143
AM161_029	AM161_034	7.173200	0.216540	-0.174286
AM161_029	AM161_035	7.123700	0.030160	-0.065714
AM161_029	AM161_036	7.153990	0.197208	-0.174286
AM161_029	AM161_037	6.028240	-0.305320	0.151429
AM161_029	AM161_038	5.306230	0.429167	-0.228571
AM161_030	AM161_031	6.929010	0.094204	-0.038571
AM161_030	AM161_032	14.841000	-0.032358	0.015714
AM161_030	AM161_033	6.416780	0.212512	-0.147143
AM161_030	AM161_034	6.389790	0.016367	-0.038571
AM161_030	AM161_035	6.281810	0.081126	-0.065714
AM161_030	AM161_036	6.369890	-0.009447	-0.092857
AM161_030	AM161_037	3.860710	-0.478864	0.260000
AM161_030	AM161_038	5.462660	0.189472	-0.092857
AM161_031	AM161_032	8.057960	0.072863	-0.120000

Individual1	Individual2	Geographic distance (km)	Relatedness	
			r (Wang 2002)	*Aij (Rousset 2000)*
AM161_031	AM161_033	5.299190	0.156174	-0.092857
AM161_031	AM161_034	5.351860	0.193832	-0.120000
AM161_031	AM161_035	5.291340	-0.149291	0.097143
AM161_031	AM161_036	5.330900	-0.144170	0.042857
AM161_031	AM161_037	4.084510	-0.160541	0.124286
AM161_031	AM161_038	3.390770	0.211318	-0.065714
AM161_032	AM161_033	12.361700	-0.128672	0.042857
AM161_032	AM161_034	12.429500	-0.084509	-0.038571
AM161_032	AM161_035	12.418400	-0.204161	0.097143
AM161_032	AM161_036	12.417400	0.232564	-0.147143
AM161_032	AM161_037	12.079600	-0.173795	0.042857
AM161_032	AM161_038	11.013700	0.188924	-0.065714
AM161_033	AM161_034	0.069787	-0.019921	-0.038571
AM161_033	AM161_035	0.139325	0.689120	-0.364286
AM161_033	AM161_036	0.067585	0.642866	-0.310000
AM161_033	AM161_037	2.923050	-0.273877	0.178571
AM161_033	AM161_038	2.067360	0.209643	-0.147143
AM161_034	AM161_035	0.111840	0.047827	-0.011429
AM161_034	AM161_036	0.025246	-0.007231	-0.065714
AM161_034	AM161_037	2.922460	-0.075991	0.070000
AM161_034	AM161_038	2.104080	0.167735	-0.120000
AM161_035	AM161_036	0.089139	0.487490	-0.255714
AM161_035	AM161_037	2.811660	-0.149686	0.178571
AM161_035	AM161_038	2.019730	0.203186	-0.147143
AM161_036	AM161_037	2.898110	-0.151434	0.070000
AM161_036	AM161_038	2.079960	0.196937	-0.174286
AM161_037	AM161_038	1.606120	-0.533333	0.260000

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Australian Centre for Wildlife Genomics Results Report

Addendum Report II

This is a second addendum to the previous report by Neaves *et al.* (2015), relating to the genetic assessment of a Koala (*Phascolarctos cinereus*) population in the Wardell area of the mid-north coast of New South Wales, which was conducted by the Australian Centre for Wildlife Genomics (ACWG), Australian Museum Research Institute and submitted to the Roads and Maritime Service (RMS) on the 12th August 2015.

Addendum details:

On the 14th October 2015, following the Koala Expert Advisory Committee PVA Workshop, RMS requested estimates of dispersal (the number of Koalas per generation; *Nm*) to be calculated using the Shannon's Mutual Information index (Sherwin et al., 2006; Rossetto et al., 2008; Dewar et al., 2011) under the assumption that dispersal was symmetrical. These analyses were to be completed by Friday the 23rd October for inclusion into the PVA undertaken by RMS.

Estimates of dispersal were requested for:

1. Between Wardell and the surrounding areas; and
2. Within Wardell, between areas East and West of the proposed upgrade

The average level of dispersal between Wardell and surrounding sites ranged from 0.199 – 0.400 individuals per generation. These levels are relatively low but reflect the geographical distance between sites. An estimate of 0.400 (per-locus range = 0.076 – 23.224, Table 1) Koalas per generation from the comparison between Wardell and Tyagarah represents the most useful estimate for the PVA, as these sites are closest geographically. Dispersal of males and females appears similar (Nm_{MALES} = 0.304 Koalas per generation, $Nm_{FEMALES}$ = 0.386 Koalas per generation, Table 2).

Within Wardell, 1.56 Koalas per generation dispersed between the Eastern and Western sides of the proposed upgrade, with an upper limit of 50.588 and lower limit 0.145 based on individual loci. Given the small sample sizes we did not assess differences between the sexes.

The details of the analyses and results are described below and a complete list of the pairwise dispersal estimates for each locus and overall is appended at the end of this report.

Yours sincerely,

Dr Rebecca Johnson, on behalf of the co-authors listed below.

Dr Linda Neaves, Siobhan Dennison, Dr Greta Frankham, and Dr Mark Eldridge

Details of Analyses and Results:

We generated locus-by-locus and overall estimates of dispersal between Wardell and surrounding locations (Port Macquarie, Coffs Harbour and Tyagarah), and within Wardell using the same samples and 17 microsatellite markers described in the original report (Neaves *et al.,* 2015). Pairwise estimates of dispersal (*Nm*) were calculated using the Shannon's Mutual Information index (Sherwin et al., 2006; Rossetto et al., 2008; Dewar et al., 2011) implemented in GENALEX (Peakall and Smouse 2006, 2012). Where possible data on the sex of sampled koalas was included, for Wardell samples this was obtained from Phillips *et al*. 2015 (20 females, 15 males, 3 unknown).

1. *Between Wardell and the surrounding areas*

Overall the estimates of dispersal in and out of Wardell were relatively low (*Nm* = 0.199 – 0.400 Koalas per generation across all sites, Table 1), but this is not surprising given the distance between Wardell and these sites. The highest level of dispersal was found between Wardell and Tyagarah (*Nm* = 0.400 ; Table 1), which are closest geographically. This is consistent with the previous genetic analysis showing Wardell and Tyagarah were genetically similar (i.e. the same genetic cluster, Figure 6 of Neaves *et al*. 2015). Given the proximity of the two sites this value represents the most useful estimate for the PVA, but the level of immigration/emigration is likely to be higher between areas closer to Wardell, e.g. Lismore.
Comparisons of male and female dispersal between Tyagarah and Wardell indicate similar levels of dispersal for females (*Nm* = 0.386) and males (*Nm* = 0.304). These estimates of dispersal are comparable to the estimate obtained overall (Table 2).

2. *Within Wardell, between areas East and West of the proposed upgrade*

Comparison of Koalas sampled East and West of the proposed upgrade indicated 1.5 Koalas per generation are dispersing, with estimates for individual loci ranging from 0.145 to 50.588. These estimates should be treated with caution given the small sample size, particularly for the Eastern side of the proposed road (*n* = 5). To assess variation in movement across Wardell and potential impacts of skewed sample sizes we assessed dispersal between eastern and western areas of Wardell using even sample sizes (rather than the proposed road). This indicated approximately 11.6 Koalas moved per generation across the site. Hence, while it is possible that dispersal within Wardell has been underestimated due to the skewed sample size it likely falls within the range reported above.

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Table 1. Locus by locus estimates of dispersal (Koalas per generation; *Nm*) between sampling locations based on Shannon's mutual information index. Overall *Nm* estimates can be found at the bottom

Port Macquarie & Wardell		Coffs Harbour & Wardell		Tyagarah & Wardell	
Locus	**Nm**	**Locus**	**Nm**	**Locus**	**Nm**
Pcin3	0.256	Pcin3	0.611	Pcin3	0.920
Pcin5	0.098	Pcin5	0.451	Pcin5	0.161
Pcin6	0.131	Pcin6	0.153	Pcin6	0.168
Pcin7	1.702	Pcin7	0.720	Pcin7	23.224
Pcin8	0.326	Pcin8	0.491	Pcin8	0.451
Pcin9	0.122	Pcin9	0.330	Pcin9	0.231
Pcin10	0.036	Pcin10	0.063	Pcin10	3.298
Pcin11	0.048	Pcin11	0.089	Pcin11	0.076
Pcin14	2.314	Pcin14	0.340	Pcin14	0.492
Pcin15	0.328	Pcin15	0.230	Pcin15	0.503
Pcin19	0.793	Pcin19	1.491	Pcin19	1.077
Pcin20	0.732	Pcin20	1.848	Pcin20	0.388
Pcin21	0.812	Pcin21	0.659	Pcin21	19.085
Pcin22	0.071	Pcin22	0.093	Pcin22	0.397
Pcin23	1.252	Pcin23	0.967	Pcin23	0.152
Mean over Loci	**0.199**	**Mean over Loci**	**0.289**	**Mean over Loci**	**0.400**

Table 2. Sex-specific locus by locus estimates of dispersal (Koalas per generation; *Nm*) between Tyagarah and Wardell based on Shannon's mutual information index, with the overall *Nm* estimate at the bottom

Tyagarah & Wardell		
Locus	**Nm (males)***	**Nm (females)#**
Pcin3	0.790	0.651
Pcin5	0.111	0.191
Pcin6	0.098	0.277
Pcin7	3.836	2.212
Pcin8	0.353	0.375
Pcin9	0.417	0.148
Pcin10	1.644	8.009
Pcin11	0.059	0.088
Pcin14	0.435	0.235
Pcin15	0.218	0.565
Pcin19	1.131	1.175
Pcin20	0.251	0.686
Pcin21	1.690	9.562
Pcin22	0.627	0.394
Pcin23	0.148	0.145
Mean over Loci	**0.304**	**0.386**

**n* = 23 (8 from Tyagarah, 15 from Wardell)

#*n = 29* (9 from Tyagarah, 20 from Wardell)

Table 3. Locus by locus estimates of dispersal (Koalas per generation; *Nm*) based on Shannon's mutual information index. Overall *Nm* can be found at the bottom

East-West of proposed road (East *N* = 5, West *N* = 33)	
Locus	**Nm**
Pcin3	1.060
Pcin5	18.753
Pcin6	44.696
Pcin7	0.145
Pcin8	23.346
Pcin9	1.607
Pcin10	8.781
Pcin11	0.621
Pcin14	50.588
Pcin15	0.236
Pcin19	3.382
Pcin20	5.389
Pcin21	1.532
Pcin22	9.528
Pcin23	0.809
Mean over Loci	**1.563**

From: Rod Kavanagh
To: RAVALLION Julie A
Cc: WILSON Simon; LAWRENCE Scott B
Subject: FW: Dispersal Estimates - additional information
Date: Tuesday, 3 November 2015 4:04:53 PM
Attachments: image001.png
image002.wmz
image003.gif

Hi Julie,

These are the "comparative" results received late on Friday from Siobhan from the Australian Museum – for your records.

Regards,

Rod

Dr Rod Kavanagh
Principal Research Ecologist
Sydney office
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From: Siobhan Dennison [mailto:Siobhan.Dennison@austmus.gov.au]
Sent: Friday, 30 October 2015 4:03 PM
To: Rod Kavanagh
Cc: Linda Neaves; Greta Frankham; Rebecca Johnson; Mark Eldridge; 'William Sherwin'
Subject: Dispersal Estimates - additional information

Dear Rod,

Here is the additional information you requested following our conversation earlier today. I have re-calculated *Nm* for both within-Wardell (East vs West of the proposed upgrade), and between Wardell and Tyagarah.

After we spoke, I had some further correspondence with Bill Sherwin too, who suggested that the arithmetic mean was the correct value to use rather than the value I had reported, which was output by Genalex. I have therefore included the arithmetic mean and standard error from these new analyses in **Table 1** below for you.

In addition, I re-calculated the arithmetic mean and standard error from the original analyses in Addendum II (calculated using the *sHua* cut-off of 0.0001) for these areas:

Between Tyagarah and Wardell, Nm = 3.375 (SE = 1.886)
Within Wardell, East and West of the proposed upgrade, Nm = 11.365 (SE = 4.215)

I hope this helps! The values in the table below seem to align more with the values you gave me from the SCU report. Please do not hesitate to get back in contact if you have any further questions.

Thank you and Kind Regards,

Siobhan

Table 1. Locus by locus estimates of *Nm* between (i) eastern and western sides of the proposed upgrade within Wardell, and (ii) between Wardell and Tyagarah (*sHua* cut-off = 0.01)

Wardell East-West of proposed road (East *N = 5*, West *N = 33*)		**Tyagarah & Wardell (Tyagarah *N = 17*, Wardell *N = 38*)**	
Locus	**Nm**	**Locus**	**Nm**
Pcin3	4.044	Pcin3	1.198
Pcin5	26.355	Pcin5	0.200
Pcin6	5.232	Pcin6	0.232
Pcin7	5.407	Pcin7	35.732
Pcin8	47.839	Pcin8	0.664
Pcin9	31.291	Pcin9	0.324
Pcin10	6.700	Pcin10	3.506
Pcin11	5.171	Pcin11	0.103
Pcin14	-*	Pcin14	0.538
Pcin15	4.275	Pcin15	0.632
Pcin19	45.999	Pcin19	1.316
Pcin20	-*	Pcin20	0.662
Pcin21	38.103	Pcin21	26.459
Pcin22	6.545	Pcin22	0.585
Pcin23	33.159	Pcin23	0.180
Mean (SE)**	**17.341 (4.538)**	**Mean** (SE)**	**4.822 (2.799)**

* no estimate could be made for this locus, because *sHua* fell below the cut-off value
** arithmetic mean

--
Siobhan Dennison
Technical Officer | Australian Centre for Wildlife Genomics
Australian Museum 1 William Street Sydney NSW 2010 Australia
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PEER REVIEW OF TWO REPORTS:

<u>"AMRI"</u>

"Koala population genetics management. A report to the Roads and Maritime Service (RMS)" 12th August 2015. Linda E. Neaves, Siobhan B. Dennison,, Greta J. Frankham, Mark D. B. Eldridge and Rebecca N. Johnson. Australian Museum Research Institute

and

<u>"SCU"</u>

"Genetic profiling of koalas: Woolgoolga to Ballina Pacific Highway Upgrade (Section 10-Wardell to Coolgardie)". August 2015. Dr J.A. Norman, Dr C. Blackmore, Assoc. Prof. R. Goldingay & Prof L. Christidis. Southern Cross University.

ASSESSMENT BY:

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UNSW DISLCAIMER

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SUMMARY and RECOMMENDATIONS

In the Wardell area of northern NSW, a planned upgrade to the Pacific Highway passes through an area inhabited by koalas. NSW Roads and Maritime Services wishes to assess the possible impact of the upgrade on the koala population, and have commissioned two genetic reports: AMRI (by Australian Museum Research Institute) and SCU (by Southern Cross University).

The question “Could the upgrade disrupt koala dispersal sufficiently to reduce the viability of the koala population?” is expressed as a series of deliverables, plus the apparent intention to follow up with a Population Viability Analysis (PVA). I presume that PVA will be used comparatively, examining chance of population persistence over multiple generations, with various levels of dispersal between sub-populations. Such comparative use of PVA is called “sensitivity analysis”.

To set the baseline for such a PVA, genetic methods can be used to assess the natural amount of dispersal between areas. The margins of areas to be investigated can be set for various reasons including habitat suitability or human alteration (eg the highway upgrade).

AMRI and SCU each used a number of different methods to assess genetic subdivision, without converting them to dispersal estimates. It is currently not possible to make a direct comparison between the results of AMRI and SCU at Wardell, because there is insufficient corresponding geographic information. This should be clarified with detailed geographic information for each individual at Wardell and immediate surrounds. However, in both studies, there was a general pattern of increasing genetic similarity at decreasing separation (“Isolation by Disatnce”), but there were no clear boundaries where one locality was genetically isolated from another.

But what is the dispersal across these boundaries? In each report only one method specifically targeted dispersal, by identifying particular koalas who might have dispersed in the most recent generation: those that were genetically assigned to a location other than the one in which they were sampled (AMRI); or those with first-order relatives in a location other than the one in which they were sampled (SCU). Only a small number of individuals were identified as potential dispersers, but it is worth noting that only small numbers are needed to halt genetic differentiation by chance processes in transmission. Thus the areas within Wardell, appear to be important for mutual support of each other, and adjacent areas.

Both studies indicated that dispersal was relatively high both between subpopulations within Wardell, and between Wardell and adjacent areas. This dispersal probably does two things: opposes loss of genetic variation within subpopulations, and forestalls immediate extinction. Therefore, a precautionary conservation management approach would be to avoid any reduction of the measured level of dispersal, pending results of the PVA-sensitivity analysis.

There are two limitations to the use of AMRI’s and SCU’s genetic dispersal estimates as base-line dispersal for the PVA-sensitivity analysis. The first limitation is that sample sizes are unavoidably small, so that the estimates are subject to high error rates. This is compounded by the fact that these particular analyses only tell us about dispersal in the most recent generation. AMRI and SCU were correct to avoid methods that convert their genetic subdivision estimates into dispersal rates, because this has been criticised on various grounds.

However, there exists a Mutual Information method that avoids the problems that beset other genetic dispersal measures, and can deal with the widest possible range of population sizes and dispersal rates. The data from AMRI and SCU should be used in this way, to produce dispersal assessments as a baseline in the PVA-sensitivity analysis, to investigate how the koala population’s viability might be affected if the Pacific Highway upgrade reduces dispersal below this baseline.

AMRI and SCU also produce estimates of Genetic variation within subpopulations, which can be included in some PVA programs.

Finally, in commissioning the analysis of sensitivity of population extinction to altered dispersal, I encourage the Roads and Maritime Services to require information on not only the most likely outcome, but also the worst-case outcome, to facilitate precautionary management.

GENERAL COMMENTS

In the Wardell area of northern NSW, a planned upgrade to the Pacific Highway passes through an area inhabited by koalas. NSW Roads and Maritime Services wishes to assess the possible impact of the upgrade on the koala population, and have commissioned two genetic reports: AMRI (by Australian Museum Research Institute) and SCU (by Southern Cross University).

The scope of the two reports appears to be slightly different. The question "Could the upgrade disrupt koala dispersal sufficiently to reduce the viability of the koala population?" is expressed as deliverables that are listed by AMRI and SCU. These deliverables are abbreviated as follows.

1. CONNECTIVITY WITHIN WARDELL. AMRI 1: Analyses of population structure and gene flow within the focal area. Also SCU 1: Is the Wardell KMP spatially structured?

2. CONNECTIVITY BETWEEN WARDELL AND SURROUNDS. AMRI 2: Determine whether the focal population appears to be connected to populations in the surrounding area. Also SCU 2: Is the Wardell KMP an important source population for surrounding areas?

3. WITHIN-LOCALITY GENETIC VARIATION. AMRI 3. Allele frequency information for the population as a whole (and for each subpopulation if relevant).

4. VALIDATION. AMRI 4. Summary of procedures undertaken for data validation.

The SCU report indicated the intention to follow these reports with a formal Population Viability Analysis (PVA). I presume that PVA will be used comparatively, examining chance of population persistence over multiple generations, with various levels of dispersal between sub-populations. Such comparative use of PVA is called "sensitivity analysis" (Penn *et al*. 2000; Reed 2009).

To set the baseline for such a PVA, genetic methods can be used to assess the natural amount of dispersal between areas. The margins of areas to be investigated in this way might be set for various reasons including habitat suitability, human alteration (eg the highway upgrade), or intrinsic differences such as genetic incompatibilities (the latter are unlikely in the case of koalas, Sherwin *et al.* 2000). In these reports, the AMRI and SCU are asked to assess dispersal between areas within Wardell KMP (deliverable 1), and between Wardell KMP and adjacent areas (deliverable 2).

The spatial arrangement of sampling within Wardell was evident in the SCU study, but not in the AMRI study – perhaps AMRI did not have access to detailed location data.

There are many different methods that use genetic data to estimate average levels of dispersal between areas for a PVA. The AMRI and SCU reports each use a number of different methods to assess genetic subdivision, most of which were not converted to dispersal estimates. In both studies, there was a general pattern of increasing genetic similarity at decreasing separation ("Isolation by Disatnce"), but there were no clear boundaries where one locality was genetically isolated from another.

In each report only one method specifically targeted dispersal, by identifying some individuals who might have dispersed in the most recent generation: koalas that were genetically assigned to a location other than the one in which they were sampled (AMRI); or koalas with first-order relatives in a location other than the one in which they were sampled (SCU).

Both studies indicated that dispersal was relatively high both between subpopulations within Wardell, and between Wardell and adjacent areas. As the authors point out, this dispersal probably does two things: opposes loss of genetic variation within subpopulations (thus potentially aiding future adaptability, Frankham *et al.* 2010), and forestalls immediate extinction, which is a risk in small isolated populations, including koalas (Lunney *et al.* 2002).

Therefore, a precautionary conservation management approach would be to avoid any reduction of the measured level of dispersal, unless there had been assessment of the potential effects of such reduction. However, if PVA-sensitivity analysis shows that a certain reduction of dispersal (x%) is not likely to significantly affect population viability, then it would be reasonable to implement particular management options, if it could be reliably demonstrated that these options would reduce dispersal by no more than x%.

SCU recommends that the genetic measures of dispersal be incorporated into the future PVA. There are two limitations to the use of AMRI's and SCU's genetic dispersal estimates as base-line dispersal for the PVA-sensitivity analysis. The first limitation is that sample sizes are unavoidably small, so that the estimates are subject to high error rates. This is compounded by the fact that these particular analyses only tell us about dispersal in the most recent generation. AMRI and SCU were correct to avoid methods that purport to assess dispersal over tens of generations, but have been criticised on various grounds.

However, there exists a Mutual Information method that can successfully assess dispersal over tens of generations, from genetic data (Sherwin 2006, 2010, Dewar et al 2011, Chao et al 2015). This method avoids the problems that beset other genetic diversity and dispersal measures, and, unlike the other methods, can deal with the widest possible range of population sizes (10 upwards) and dispersal rates (from one in a thousand, to one-third of the population, per generation; Sherwin 2006). All the data from AMRI and SCU should be analysed by the Mutual Information method, to produce robust multigeneration dispersal assessments; the Mutual Information calculations can be carried out in the freeware GENALEX (http://biology.anu.edu.au/GenAlEx) which was used for other tasks by AMRI. The GENALEX website also contains a guide for conversion of Mutual Information for microsatellites to a dispersal estimate. For the mitochondrial DNA, such a conversion could be achieved by following either Dewar (2011, equation 8) for each variable site, or Chao et al. (2015 supplement equations B5-7) for entire haplotypes.

These dispersal assessments from Mutual Information could then be used as baseline in the PVA-sensitivity analysis to investigate how the viability of the koala population might be affected if the upgrade of the Pacific Highway reduces dispersal below this baseline level. The PVA-sensitivity analysis should assess the effect of the highway upgrade, including any measures likely to increase or decrease the road corridor's permeability to koalas, such as fences or overpasses.

The other data that can be included in the PVA is AMRI's and SCU's estimates of genetic variation within subpopulations, which can be included in some PVA programs. Its inclusion will add to realism, especially allowing assessment of when the genetic diversity at Wardell might fall below the lowest levels listed in populations of koalas and other species, reviewed by AMRI.

Finally, in commissioning the analysis of sensitivity of population extinction to altered dispersal, I encourage the Roads and Maritime Services to require information on not only the most likely outcome, but also the worst-case outcome, to facilitate precautionary management.

DETAILED COMMENT – AMRI REPORT

SAMPLING THE LOCALITIES AND INDIVIDUALS:

From Wardell there were 38 samples for microsatellite and mitochondrial DNA. The lack of identification of position for East and West Wardell makes it difficult to assess their importance relative to the proposed highway upgrade, and to compare this to the SCU report, which uses other nomenclature. Also the tiny number of samples from East makes any conclusions weak.

Four other sites near the NSW/Queensland border (Macquarie, Coffs Harbour, Tyagarah and Coomera) were sampled for both microsatellite and mitochondrial DNA, plus a sample set from the whole koala range for mitochondrial DNA only.

THE GENES USED AND THEIR VALIDATION – DELIVERABLE 4.

1. Microsatellites – biparental inheritance

The analysis was based on a good number of genes -15, and appeared suitable for the tasks of determining variability within location, and differentiation and dispersal between locations. The genes were checked carefully. Two other microsatellite genes were excluded for good reasons, and 10% of individuals were independently re-genotyped. The probability of two individuals having the same microsatellite profile was low (10^{-15}), showing that a good battery of genes had been analysed. Variants at the 15 genes appeared to be inherited independently, so that each provided useful information for the analysis (no "linkage disequilibrium"). In most cases there was no evidence of non-random mating within site (ie, there were few cases of genes out of "HWE").

2. Mitochondrial DNA – maternal inheritance

An 800 bp portion of the mitochondrial DNA control region was sequenced, and appeared suitable for the tasks of determining variability within location, and differentiation and dispersal between locations.

WITHIN-LOCALITY GENETIC VARIATION – DELIVERABLE 3.

Microsatellite diversity within locations was summarised by a suitable array of measures: allelic diversity, allelic richness, private alleles (AP on page 6, called Pa later in Table 1) expected and observed heterozygosity, Hardy-Weinberg equilibrium (HWE–Fis Table 1) and linkage disequilibrium. These measures were not out of the ordinary for koalas (Table 1).

For mitochondrial DNA, within-locality variation was assessed by suitable statistics - haplotypic diversity and nucleotide diversity - within Wardell and the four other main sites. Wardell values were not out of the usual for koalas, although 37 out of 38 individuals had the same mitochondrial genotype (haplotype) at Wardell.

CONNECTIVITY WITHIN WARDELL _DELIVERABLE 1.

And

CONNECTIVITY BEWTEEN WARDELL AND SURROUNDING AREAS – DELIVERABLE 2.

1. Microsatellites – biparental

Microsatellite geographic structure was assessed by a number of suitable methods: STRUCTURE, DAPC, F-statistics, AMOVA, Isolation-by-distance tests in Mantel, Spatial autocorrelation of pairwise relatedness in GENALEX 6.5. Many analyses were presented without saying which type of gene (microsatellite or mitochondrial) they were based upon; I believe that in all such cases, they were microsatellites.

The authors avoided specifying definitive management units, which I consider to be wise given the relatively low differentiation indicated by most measures. There was only one genetic cluster at Wardell (deliverable 1), and gradually increasing differentiation with distance from Wardell, but no sharp breaks, a pattern called "Isolation by Distance" (deliverable 2).

As well as the DAPC, there was also a PCA presented on P11 (Fig 3), but not described in the methods section. This appears to be an analysis of microsatellite data, though that is not stated. The PCA showed that the five koalas from "East of focal area" were scattered amongst those from the west of focal area. The text states that the data in Fig 3 come from within Wardell. Thus "East of focal area" appears to mean the East part of Wardell itself, rather than an area to the east of the Wardell area, which would be the interpretation in other parts of the document, where the whole of Wardell appears to be referred to as the "focal area". Perhaps for the purposes of the PCA, the "focal area" means the proposed upgraded highway. If that interpretation is correct, then there appears to be no justification, at least with this small sample, for considering the koalas on either side of the proposed upgraded highway to be members of distinct separate populations. This should be clarified with detailed geographic information for each individual, so that there could be direct comparison with the results of SCU, which is currently not possible.

There were only two exceptions to the pattern of low differentiation, but I would not prioritise these two findings over the general consensus that there is little geographic differentiation within Wardell or between Wardell and other populations). Fst and Phi-st did show significant departures from zero, but there are many criticisms of Fst, and only partial fixes for these criticisms (Sherwin 2010, Wang 2015). Phi-st likely suffers from many of the same problems as Fst, because Phi-st is also a variance partition and an "order 2" diversity measure (Hill, 1973), the two characteristics that are at the root of Fst's many problems.

Of course, low differentiation may be due to high dispersal, and some of these measures (including Fst) can be converted to measures of dispersal, but the authors wisely did not do so, given the criticisms mentioned already.

However, there was one assessment of dispersal in and out of Wardell by microsatellites. Microsatellite DNA is biparentally inherited, so it traces dispersal of both sexes. Microsatellites were used to assess dispersal by identifying some individuals who might have dispersed in the most recent generation: koalas that were genetically assigned to a location other than the one in which they were sampled. The assignment test used was in GENALEX 6.5. It showed that some individuals were likely to have moved between Wardell and nearby localities such as Coffs Harbour, but the authors noted that the conclusions were hampered by a lack of samples from localities immediately adjacent to Wardell. Only a small number of individuals were identified as potential dispersers, but it is worth noting that only small numbers are needed to halt genetic differentiation by chance processes in transmission (Kimura and Crow, 1970).

2. Mitochondrial DNA – female dispersal

Mitochondrial DNA generally confirmed the results of the microsatellite analysis, but indicated slightly reduced dispersal of females, relative to males

Mitochondrial DNA is maternally inherited, so traces female dispersal. Mitochondrial geographic structure was assessed by suitable methods: AMOVA/Phi-ST versus distance, and a haplotype network. The AMOVA showed that 92% of mitochondrial variation was within locations. This contrasts with 75% of biparentally-inherited microsatellite variation being within locations, suggesting limited female dispersal. However, Wardell mitochondrial haplotypes do occur elsewhere, so that there must be some female dispersal. Also, mitochondrial DNA suggested that one individual was an immigrant to the Wardell area.

DETAILED COMMENT – SCU REPORT

SAMPLING THE LOCALITIES AND INDIVIDUALS:

Tables 1 and 5 show 47 samples sourced from the Wardell KMP plus two adjacent localities to the north: Lynwood and Dalwood. This group of samples will be collectively referred to as "Wardell" throughout this assessment. There were also an additional 88 koalas outside Wardell, whose locality information was somewhat scattered in the document, but from Figure 4 it seems that there were three sample sets from localities immediately to the west of Wardell, named from north to south as 30 koalas from "North Lismore", 20 from "South Lismore" and 22 from "Western". There were also 16 other koalas from further to the west of Wardell ("Far-western").

THE GENES USED AND THEIR VALIDATION – DELIVERABLE 4 (not listed as such by SCU).

The SCU analysis used only microsatellite genes - fourteen of them, and adequate number. It is not clear if this set of genes overlaps the set of genes analysed by AMRI. The variation at these genes was sufficient to give a 99% chance that a non-parent would be excluded as a potential parent. Microsatellite analysis of one known parent-offspring pair gave a relatedness estimate of approximately 0.5 (the correct value for such a pair. These values give confidence to the subsequent work assigning first-order relatives (FOR – parent-offspring or full-sibling). It was stated that these microsatellites are "able to detect the presence of genetic differentiation amongst populations with a power of 0.975 or higher after 10 generations and assuming an effective population size of 50-200". It was not explained how this power analysis was carried out.

WITHIN-LOCALITY GENETIC VARIATION – DELIVERABLE 3 (not listed as such by SCU).

Microsatellite diversity within-locations was not unusual for koalas (Table 5). In the north part of Wardell, there was marginally higher genetic variation than in the south (Table 5, with no confidence limits, so the significance of the difference cannot be evaluated). In the north part of Wardell, there was also lower mean relatedness (Table 3). If real, these two differences could indicate that the north has larger population size, or that it receives more immigration from elsewhere.

CONNECTIVITY WITHIN WARDELL _DELIVERABLE 1.

<u>Genetic Subdivision</u>

There appears to be mild genetic substructure within the Wardell area, but no complete isolation.

On Page 10 it is stated that "Genetic neighbourhood size in the Wardell KMP was estimated to be 21-30 Km^2. This confirms a pattern of limited dispersal across the study area and the likely presence of multiple subpopulations." A genetic neighbourhood is the size of an area within which mating appears to be random. Fig 1 indicates that the Wardell area is about 6km x20km, so that multiple neighbourhoods a few km across could indeed fit into the Wardell area. However, note that neighbourhood calculations are based on the idea that the population is continuous over a much larger scale than the neighbourhood, so they give no indication of sharp boundaries – indeed they assume that no such boundaries exist.

Sharp boundaries were also not supported by the FOR analysis, which suggested that connectivity between localities within Wardell is greatest between the closest localities, and decreases with distance (called "Isolation by Distance" page 10, paragraph 2).

Fst and a related quantity Rst suggest subdivision within Wardell. These measures of genetic differentiation are relatively high between North and South Wardell, compared to their values for differentiation between Wardell and the two closest localities (South Lismore and Western) west. However, these values are presented with neither significance testing, nor confidence limits. I suspect that the latter would be so wide that the comparison is meaningless - Fst has poor statistical properties, as discussed above.

On page 12 it is stated that "We also reject a model in which the Wardell KMP is divided into an eastern and western subpopulation corresponding to the two large tracts of remnant schlerophyll woodland and forest". It should be clarified why this model was rejected, and where on the map are the two large remnant tracts. It is also not clear how to compare this result to AMRI's "East" and "West", but if the division is the same in the two reports, then AMRI's finding in their PCA would confirm the SCU assertion. However, the correspondence cannot be known until the geographic and genetic data for both studies are plotted on a single map.

Genetic Estimates of Dispersal

Only one method specifically targeted dispersal, by identifying some individuals who might have dispersed in the most recent generation: koalas with first-order relatives (FOR) in a location other than the one in which they were sampled. Fig 3 showed that first-order relative pairs were found to be shared between most parts of Wardell , though decreasingly so at greater distances. Most pertinently for the purpose of the study, on pages ii and 10 it is stated that the FOR analysis confirmed that dispersal occurs across the proposed highway upgrade, at two places: Bagotville in the south, as well as in the north.

CONNECTIVITY BEWTEEN WARDELL AND SURROUNDING AREAS – DELIVERABLE 2.

Similarly to the pattern within Wardell, there is some evidence that localities more distant to Wardell are more genetically differentiated from Wardell (Table 4, again without significance testing or confidence limits). This is also reflected in the pattern of inferred dispersal events (Fig 4).

The authors also suggest that dispersal into the northern Wardell area is indicated by its relatively high levels of genetic variation (Table 5), however, as discussed above, the difference is marginal and has no confidence limits to allow assessment of its significance.

It was asserted several times that dispersal was asymmetrical, but few data were available to confirm this. There are programs such as MIGRATE that can attempt to fit models of asymmetric dispersal to genetic data, but I suspect that these programs would fail to converge, due to lack of data. With the existing smaller dataset, it might be possible to infer directionality of dispersal from the FOR data, if (1) there are data on ages of members of each FOR pair, (2) it is assumed that the younger member of the pair is an offspring, and (3) it is assumed that offspring are more likely to disperse. These assumptions mean that such an analysis might have only dubious value.

OTHER

Page 17 talks of "the potential significance of the Southern subpopulation of the Wardell KMP as the remaining relatively pure gene pool for koalas in this region". It is not clear how genetic purity is defined, nor is it explained why genetic purity is needed. Generally, the opposite - higher genetic variability - is good for conservation management (Frankham et al 2010), unless there are problems of genetic incompatibility between different races of koalas, which no-one has every suggested, to my knowledge.

Page 17 says that the south part of Wardell is more like a "functional koala metapopulation" than the north. There are two definitions of "metapopulation" (Levins 1969, Hanski 1999), either of which could probably apply to both north and south Wardell. I recommend that this term should not be used without further explanation.

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27 September 2015

Assessment of Koala genetics reports for NSW Roads & Maritime services report.

The two reports, Neaves et al. and Norman et al. each report on genetic diversity and structure of koala populations within the Wardell koala metapopulation and in relation to other regional populations. Both generate and analyse microsatellite datasets for a set of samples provided by RMS, but that is about where the congruence across project ends.

I am satisfied by the technical quality of each analysis. Norman et al. use published microsatellite loci (which ones, ref?), whereas Neaves et al. appear to have generated new marker loci. Neaves et al. use technical replicates and explicitly mention use of positive and negative PCR controls to directly assess consistency and potential for cross-contamination. No mention of this is made in the Norman et al. report. That said, based on prior experience, I do trust the quality of data provided both groups.

The scale of sampling differs between the two groups. Neaves et al include just the 34 RMS samples from the Wardell KMP, whereas Norman et al. supplement these with other samples from this region. At broader scale, Neaves et al. compare Wardell with more geographically distant populations, whereas Norman et al. have finer-scale sampling across the NE NSW region. Based on the information provided by Norman et al., most samples provided are from north or west of the proposed highway upgrade – there are very few from east of the Highway. The corollary is that the power to directly model the potential impact of the road works is rather limited.

The analytical methods differ across studies, as do their conclusions. In all cases, the methods are applied appropriately. Both studies find relatively high genetic diversity in the Wardell KMP, but superficially they come to different findings about structuring and dispersal within the region. Neaves et al. focus on summary statistics (Fst; spatial autocorrelation), clustering (PCA, STRUCTURE) and assignment methods and conclude that there is little evidence for substructure within Wardell and at the larger regional scale (to SE Qld.). By contrast, Norman et al. focus on relatedness estimates, especially distances among inferred First-order relatives as a surrogate for dispersal. They conclude that there is local structuring, with a local genetic neighboohood size of ~ 30 km^2 but, paradoxically, also infer a high rate of dispersal to nearby regional populations around Lismore.

These marked differences reflect the different forms of analysis, as well as scales of sampling. The Neaves et al. approach will be strongly influenced by long-term average metapopulation dynamics, possibly including colonization and density changes accompanying anthropogenic changes to habitat structure across the region (as discussed in Norman et al.). By contrast, the focus on first-order relatives by Norman et al. is better suited to analyzing recent (1-2 generation) dispersal

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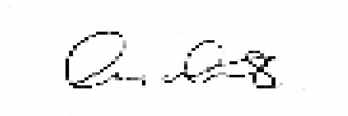
pattern, as is the finer-scale sampling across the region. Accordingly, I find the results of Norman et al. more relevant to the question at hand.

That said, I do have some reservations and recommendations:

1. The rather poor sampling east of the proposed new road limits the power to test directly for potential disruption of connectivity.
2. The results in Norman et al. are somewhat counter-intuitive in suggesting higher contemporary dispersal rates at large than small scale. Their analyses do not infer the direction of dispersal, so whether the southern populations are a source for those to the west remain speculation.
3. To address (1) in the context of the forthcoming PVA, it should be possible to model dispersal rate (using logistic regression on FOR distances or regression of pairwise r values) as a function of linear distance and habitat heterogeneity and with or without a potential road barrier
4. To address (2), methods that infer migration rates over recent generations (e.g. BayesAss) could used to test for asymmetry among north, south and adjacent regional populations.

Yours truly,



Appendix 4 – RMS discussion paper on proposed Koala connectivity structures

27 November 2015

Draft Discussion Paper
Woolgoolga to Ballina Upgrade
Proposed Koala Connectivity Measures
for
Section 10 (Broadwater to Coolgardie)

Background

The Woolgoolga to Ballina Project was approved by the State Government in June 2014 and the Federal Government in August 2014 subject to very specific conditions of approval.

Koala Connectivity is a key component in both approvals, particularly between Richmond River, Wardell and on to Coolgardie Interchange (known as Section 10).

The Federal Approval conditions for Section 10 require Roads and Maritime (RMS) to prepare and submit a Ballina Koala Plan for consideration and approval 3 months prior to construction in Section 10 and that a population viability modelling must be undertaken on the Ballina Koala population over a period of no less than 50years taking into account the impacts resulting from the road upgrade in Section 10.

While the approved route is located in mostly cleared land RMS is committed to making sure the koala and other species can safely cross under or over the new highway.

This draft discussion paper outlines the proposed mitigation measures as input into the population viability analysis being undertaken by Dr Rod Kavanagh.

Proposed Mitigation Measures

Attachment A describes the refinement of proposed mitigation measures for Section 10 over time:

- The release of Environmental Impact Statement (EIS) for the Woolgoolga to Ballina project in December 2012
- The release of Submissions and Preferred Infrastructure Report (SPIR) for the Woolgoolga to Ballina project in December 2013
- Additional measures announced by the Minister for Roads, Maritime and Freight in June 2014 and reaffirmed in January 2015. The announced additional measures included
    - Fully fencing nearly 16 kilometres of both sides of the new highway which will be connected to the fauna crossing structures.
    - Increasing the number of fauna crossings suitable for koalas by more than 400 per cent to that proposed in the December 2013 SPIR (from

six to approximately 25 structures by increasing the size of the drainage structures for use by koalas).

- Construction of a land-bridge (at least 30 metres wide) north of the Richmond River crossing, south of Bagotville.

- Planting some 130 hectares of koala food trees on RMS owned land near the new highway corridor where at least 50 per cent will be planted prior to construction and the remainder after construction.

  It was noted more land may become available for planting as RMS completes the property acquisition for this section for the highway upgrade.

- Further proposed design refinements by the RMS in June and July 2015 following further feedback from experts and the project teams

Proposed Design Refinements for Section 10

The following summarises the proposed mitigation measures for the new highway upgrade

(a) Fully fencing the highway corridor

Providing koala fencing on both sides of the new highway as well as installation of koala roller grids to provide a 'closed system'

Fig 1. Koala Floppy top fencing installed on the Pacific Highway Bonville Upgrade- shown to be effective at preventing koalas accessing the highway.

Fig 2. Example of proposed koala roller grids to be installed

-

(b) Improved Connectivity Structures

As part of the further design refinements to provide additional and improved connectivity, RMS and its project team raised and rolled the gradeline of the new highway. The current revised gradeline and its comparison to the SPIR has been included as Attachment A, the additional earthwork quantities have been include in the costing spreadsheet included as Appendix B.

A series of workshops have been held with the expert panel and government agencies to go through the PVA progress and to highlight various mitigation measures strategies including the modified gradeline and inclusion of additional crossing structures.

There was general discussion on the merit of land bridges (fauna overpasses) vs the use of plank bridges in terms of which gives the best connectivity result for the koala for the same level of investment . A list of issues with Landbridges vs Plank Bridges are listed in Table 1

Table 1 – Land bridge vs Plank Bridges

Land bridges	Plank Bridges
Provide a single point of connectivity	Can provide multiple connectivity locations
Long construction period	Short construction period
Large construction impact e.g. significant volumes of material need to be removed and placed along the alignment	Minimal construction impact confined to plank bridge area
Long establishment time for growth on landbridge 2+ years	Existing growth abutting bridge can mostly be retained
Koala Connectivity may take longer	Koala Connectivity obtained upon opening of bridge

.
At the workshop with the expert panel and agencies on the 13 July 2015 it was agreed in principle that the provision of plank bridges and additional culverts in key koala hot spots provided better Koala Connectivity then the current proposed Landbridge and this will be tested in the PVA. The various refinements discussed are included as Attachment A . In conjunction with the removal of the Landbridge and the associated savings it has been possible to replace some of the 2.4x2.4 and 3.0x3.0 RCBC's with plank bridges and introduce new plank bridges in key koala hotspots and areas proposed for koala revegetation. Plank bridges provide enhanced fauna connectivity to RCBC's as can be seen in the detail below the openness of the plank bridges will facilitate the movement of koalas and are proven to be effective for koala connectivity.

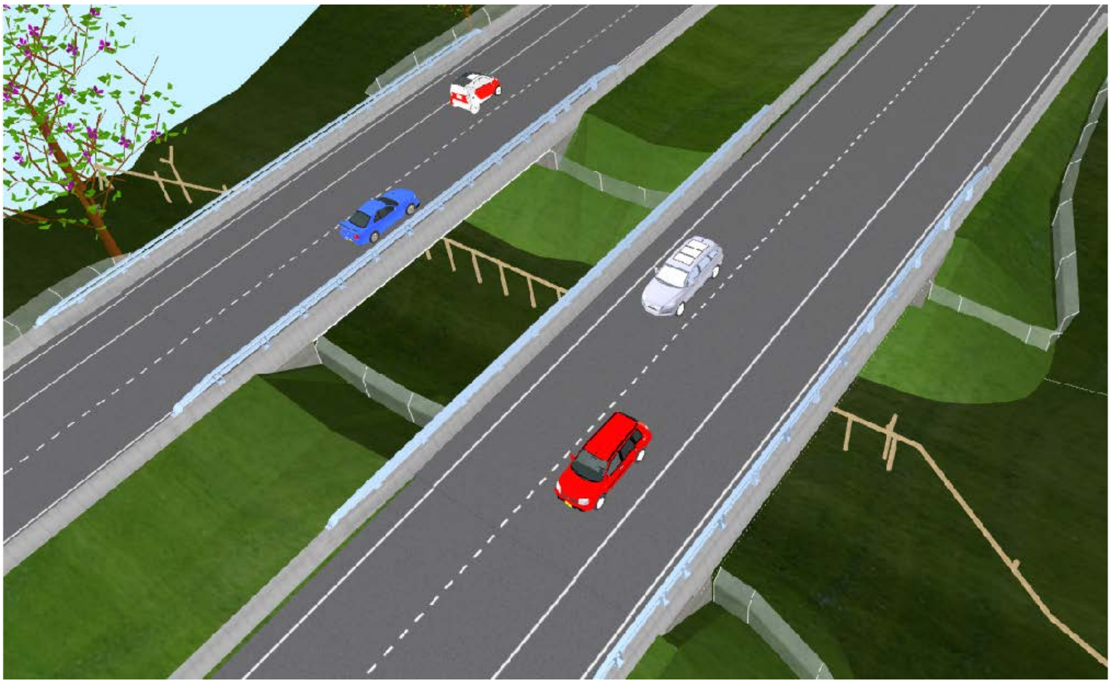


Fig 3. 3D Model of typical 15m plank bridges for koala connectivity- note twin bridges and daylighting in the median

Fig 4. 3D Model of typical 15m plank bridges for koala connectivity

Fig 5: *Fauna Underpass bridge at Pacific Highway Bonville Upgrade . . Results from AMBS monitoring have shown koalas using this underpass*

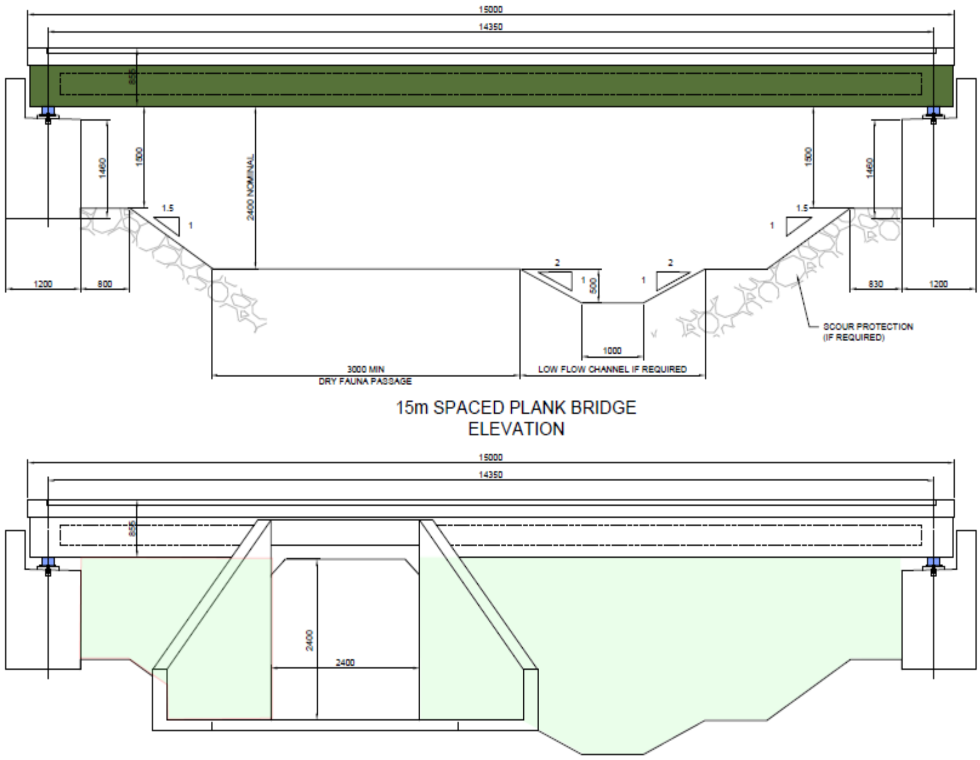


Fig 6: Comparison of culvert openings to plank bridges.

c) Additional Planting of food trees for koalas

RMS has purchased over 150 hectares of private land and has developed a revegetation strategy to transform 130 hectares of currently cleared land to koala habitat. Planted area will be protected in perpetuity through a conservation covenant mechanism. The areas of planting are shown below along with the location of Koala friendly crossings. Planting is planned to commence in Autumn or Spring next year.

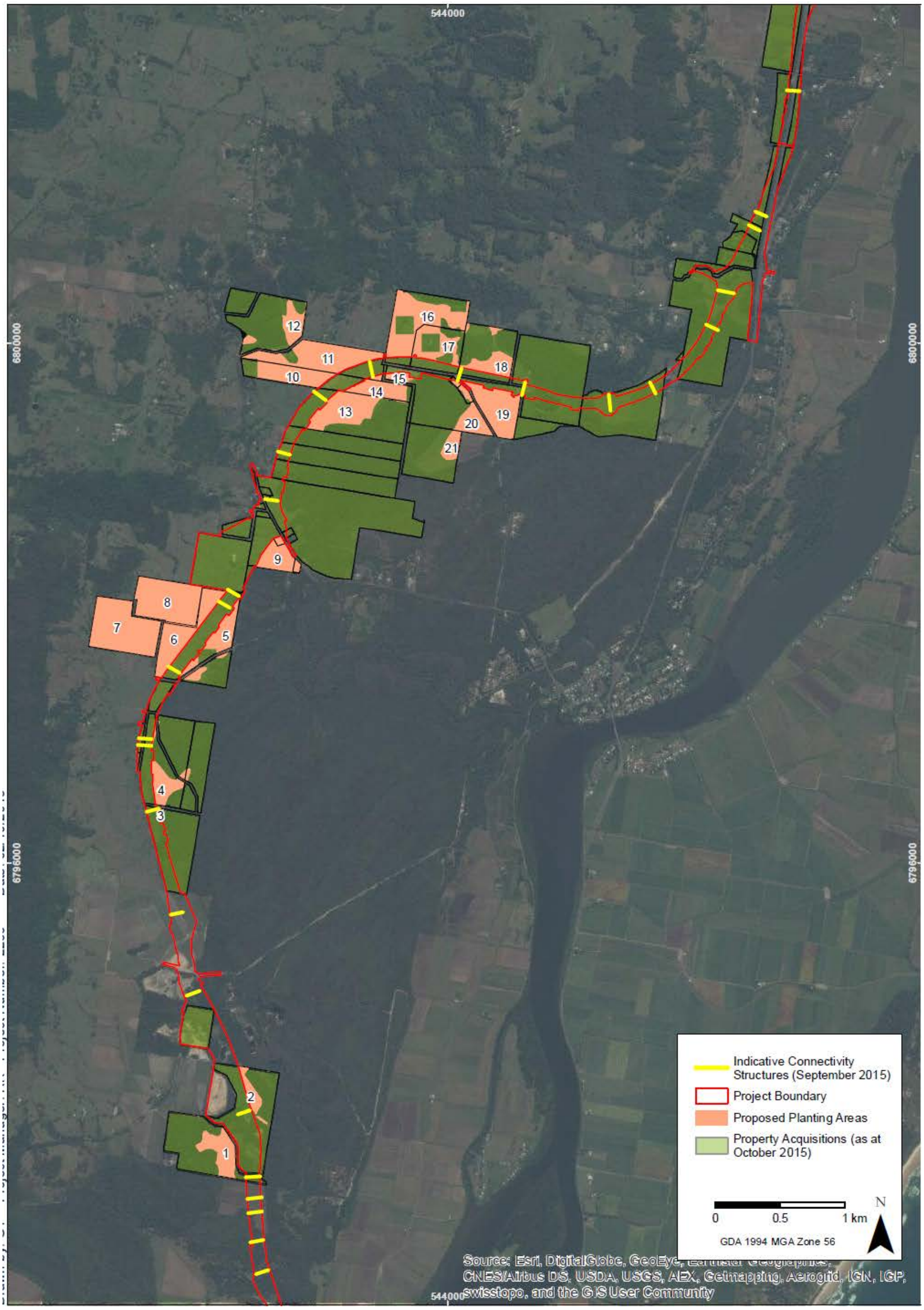


Fig 7. Proposed koala revegetation areas shown in pink shading. Source Niche Environment & Heritage

d) Maintaining connectivity during construction

The Minister for Roads and Freight committed to the construction of a land-bridge North of the Richmond River crossing. In conjunction with this, MCoA D9(f) states, “provision for the installation and vegetation planting of fauna overpasses prior to the commencement of construction".

As discussed in section (b) above, through the progression of the Koala Management Plan and Population Viability Analysis (PVA) a series of workshops have been held with koala experts and government agencies to go through the PVA progress and to highlight various mitigation measures including modified gradeline and inclusion of additional crossing structures. As a result the land-bridges have been removed and replaced with a number of plank bridges in koala connectivity hotspots and therefore the land-bridges can no longer be constructed prior to construction. An alternative approach to constructing connectivity structures early is to adopt the same connectivity strategy that is proposed for the emus in section 3, which allows koala connectivity across the construction corridor during out of hours on all proposed plank bridge locations within the koala hotspots of section 10 (south of chainage 154400). Koalas are mostly nocturnal and therefore this approach immediately allows koalas to move across the construction corridor at night time. This is an improvement from constructing structures early because it provides 8 crossing points for the duration of the construction lifecycle.

- All bridges will be constructed progressively across Section 10. As soon as each bridge is completed it will be tied in with the koala exclusion fence and site remediation completed to open up the crossing zone.
- Prior to and during bridge construction temporary fencing would be used to develop an koala passageway or race to direct koalas across the entire width of the construction corridor. The race would be established perpendicular to the corridor. Where there is a creek the race would be constructed along the creek and incorporate riparian habitat either side of the top of the creek bank. Where flatter and wider creeks occur, the area of the creek profile would also be retained inside the race. Where there is no creek, the race should be a minimum of 5 metres wide and set up through the centre of the crossing zone where possible.
- There will be a total of 8 koala races established associated with the plank bridges between Richmond River and chainage 154400. At the start of each work day temporary gates at either side of the race would be closed and then re-opened at the end of each work day. These gates would then also remain open during non-work days such as Sundays, wet days and public holidays.

e) Construction Mitigation Measures

RMS has also developed Koala Management Plan for other Sections of the Woolgoolga to Ballina Upgrade these are also relevant to Section 10 and are summarised below:

- Pre-clearing surveys to identify Koalas within the construction corridor.
- Identification of exclusion zones and fencing to prevent damage to native vegetation and Koala habitat.
- Siting of ancillary facilities to avoid impacts to known and potential Koala habitat.
- Implementation of a dog policy to ensure that no domestic dogs are brought onto the site.
- Induction and training of construction staff to make them aware of Koala habitat requirements, clearing extents and no-go areas. This training would identify areas of Koala habitat, crossing zones and key threats to the species. The importance of following the clearing and rehabilitation protocols would be made clear to all project personnel.

- Clearing of trees will be undertaken in a way that ensures Koalas living in or near the clearing area have enough time to move out of the site without human intervention. In summary this involves:
- Staged clearing, i.e. sequential thinning or partial removal of trees in progressive stages, to allow Koalas to safely leave the clearing area and relocate to adjacent habitat.
- An ecologist will undertake surveys of the scheduled clearing area prior to vegetation clearing (i.e. early in the morning prior to the commencement of vegetation clearing activities) to identify trees in which a Koala is present and any adjacent trees with overlapping crowns.
- Suspension of clearing works for a minimum period of 48 hours if a Koala is found within a clearing area to allow the animal to move out of the construction site on its own volition.
- The direction of sequential clearing will be away from threatening processes or hostile environments, i.e. roads. The ecologist is responsible for verifying that sequential clearing has taken place.
- Each tree identified by the ecologist as being a risk to a Koala if felled, will not be felled, damaged or interfered with until the Koala has moved from the clearing site. The ecologist will physically move Koalas if necessary in accordance with Biodiversity Guidelines: Protecting and managing biodiversity on RTA Projects (RTA 2011).
- In the event that a Koala remains in the clearing site for more than 48 hours, it will be captured and translocated by a suitably qualified person to the nearest area of habitat identified as suitable for Koala release and where the individual is at no risk of further harm.
- An ecologist will be present on site prior to and during all vegetation clearing to allow Koalas to safely leave the clearing site and relocate to adjacent habitat without human intervention. In the event that a Koala does not move on its own volition after a period of two nights, it will be trapped. The 'corflute method' would be used for trapping Koalas. This typically involves the use of a plastic guard, or similar material (approximately 100 centimetres tall) and, optionally, a cage trap arrangement placed in the fence near the base of the target tree, as shown below
- 

Fig 7. Trap designed to capture koalas (source AMBS 2011)

- Once captured, the Koala's health will be assessed and details recorded of age, sex, weight, body measurements, and presence of pouch or back young (for females). All healthy animals will be ear tagged, micro-chipped (using a PIT tag) and relocated into adjacent habitat identified for Koala release. Release points will be not more than 100 metres away provided that suitable habitat is present. If an injured Koala is captured, it will be transported to an experienced wildlife veterinarian for treatment. Details of veterinarians will be provided in the FFMP. The NSW Code of Practice for Injured, Sick and Orphaned Koalas (OEH, 2011) (refer to Appendix C) will be followed for trapping and relocating Koalas and dealing with any injured Koalas encountered during the clearing procedure.

- Direct interactions with Koalas must only be conducted by a suitably qualified and experienced ecologist who holds the necessary capture and handling permits issued by the OEH, or other licensed wildlife carers.
- Areas where Koalas have been captured will be recorded for consideration of inclusion as a monitoring site
- A licensed wildlife carer/ecologist will be present on site during all vegetation clearing and habitat removal activities to redirect Koalas that may be encountered during clearing activities.
- Following the clearing works and throughout the remainder of the construction period, any observations of Koalas in the construction corridor will also follow the unexpected threatened species find procedure (RTA 2011).
- All construction vehicles will be required to comply with the speed limits set out in the CEMP and to remain within the designated construction corridor. The speed limit within the construction zone will range from 10 km/hr – 60 km/hr, depending on construction activities and construction machinery. Speed limits will be reduced to 80 km/hr on the existing Pacific Highway and 40 km/hr on local access roads.
- Given the likely increased traffic on local roads during the construction period, Koala awareness signs will be erected on local roads in potential road kill areas to make motorists aware of the potential for Koalas to cross the road and the need to restrain dogs, particularly between the hours of 6 pm and 6 am when Koalas are most active. Koala awareness signs will also to be constructed along the highway upgrade at locations in close proximity to the fauna crossing zones. Signage locations will be identified in the Koala Fencing Strategy

RMS is also considering whether translocation of Koalas under the construction footprint along with monitoring of translocated individuals is a suitable mitigation measures.

Appendix 5 – Friends of the Koala, Lismore and RMS Map of indicative Koala road-kill hotspots

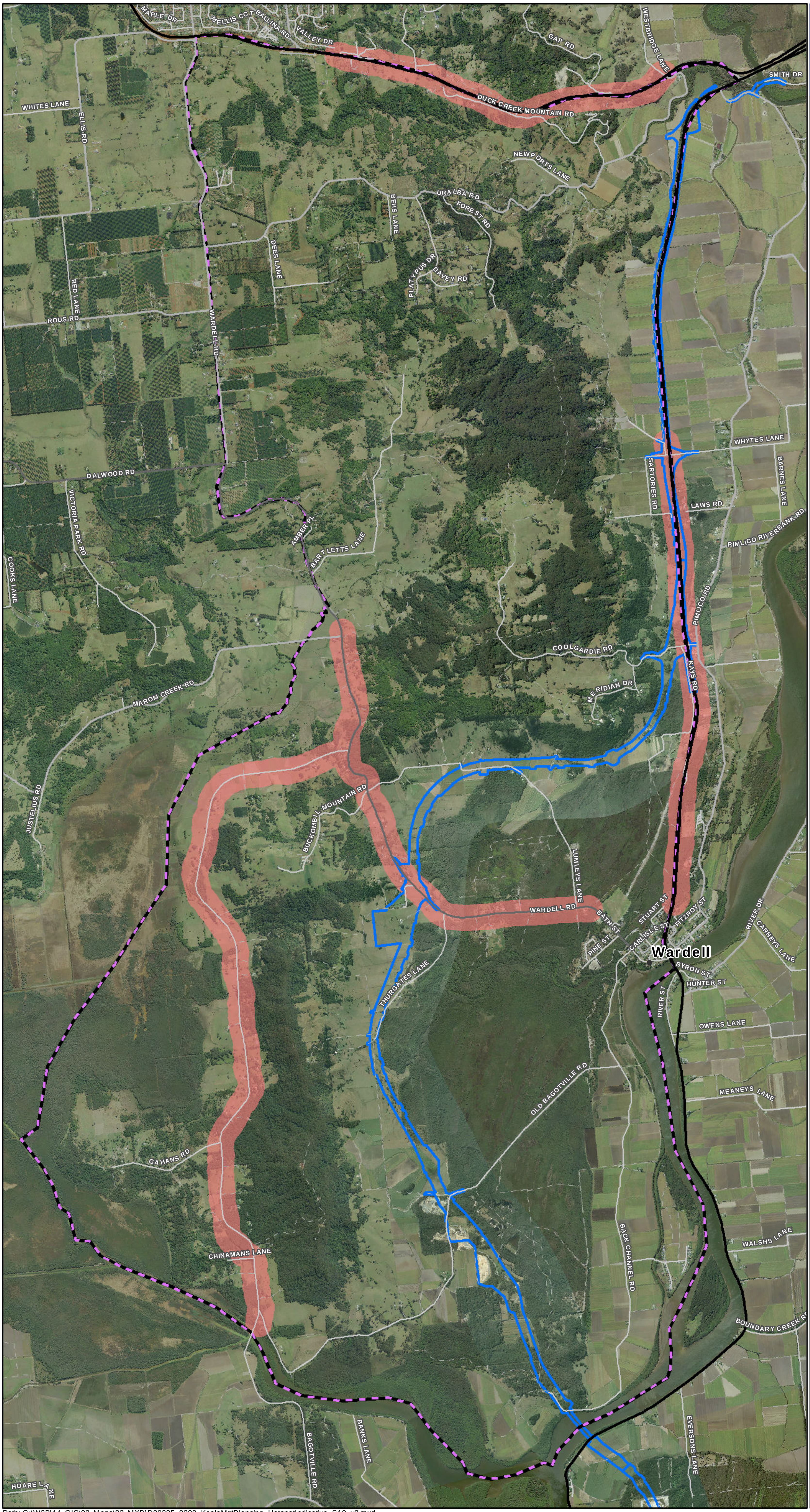
Transport
Roads & Maritime
Services

PACIFIC HIGHWAY UPGRADE

Woolgoolga to Ballina

Southern Koala Management Precinct Indicative Koala road kill hotspot Section 10

Map 1 of 1



PIR Control Line and 500m Chainages

EIS / SPIR Project Boundary (Nov 2013)

Southern Koala Mgt Precinct (11/03/2014)

Indicative Koala road kill hotspot (9/12/2015) Source: Friends of the Koala and BioLink Ecological

Cadastre

NPWS Reserves

State Forests

1:42,500
at A3

0 250 500 1,000 1,500 2,000 2,500
Metres

Print Date:	16/12/2015
Author:	SPALDINC

Map produced by Pacific Highway Branch, Infrastructure Development, RMS. Map data copyright 2015 Roads & Maritime Services, NSW. Spatial data used under licence from Land and Property Management Authority, NSW © 2015.

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APPENDIX 4 PEER REVIEW REPORT

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

School of Geography, Planning and Environmental Management

Dr Jonathan Rhodes
Associate Professor

Bob Higgins
General Manager, Pacific Highway
21 Prince Street
GRAFTON, NSW 2460

20th January 2015

Dear Bob,

I have now reviewed the Ballina Koala Plan that details the outcomes of the koala population viability analysis (PVA) of the proposed Pacific Highway upgrade near Wardell, NSW and I am happy to endorse the contents of that report. Within the constraints of limited data and the time frames within which the report had to be produced, I believe that this report is scientifically sound. The report details estimates of the potential impact of the road upgrade on the koala population in the region and identifies the extent to which habitat restoration may offset these impacts. It then considers other potential management strategies, which could further compensate for the impact of the road upgrade, by reducing mortality on other roads, or by increasing fecundity through measures such as disease management. It also assesses the robustness of the estimates of the impact of the road.

As with all models, the models underlying PVA are only as good as the data used to develop them and the appropriateness of the assumptions made in the model structures. PVA generally requires large amounts of data over many years to reliably parameterise the models that underpin PVA. In particular, reliable estimates of demographic rates and their variability through time are very hard to obtain without large sample sizes and many years of data. However, extensive data of this kind are rarely available (as is the case with this study) and therefore the reliability of absolute estimates of risks of extinction are generally highly unreliable. However, even when absolute estimates of extinction risk are unreliable, it has been demonstrated that *relative* measures of extinction risk or population size predictions will tend to be much more robust. Consequently, using PVA to compare the likely relative outcome of alternative management scenarios is a much more reliable use of PVA than using it to meet targets based on absolute measures of extinction risk. Consequently, this report focuses on comparing predicted outcomes among alternative scenarios (e.g., the impact of the road upgrade is estimated based on the difference between scenarios with the road upgrade and scenarios without the road upgrade) rather than on absolute predictions of extinction risk or population size.

Given considerable uncertainties about the input parameters for the model underpinning the PVA and the appropriate structure of that model, estimates of the impact of the road upgrade are uncertain. These uncertainties translate into a range of plausible outcomes being presented for the impact of the road upgrade, rather than a single value. Based on

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a consideration of a range of values for parameter inputs, the impact of the road upgrade is estimated to have a plausible range between having no effect to having a small affect equal to around a 10% decline in population size relative to the no road case (assuming that fencing will completely prevent mortalities on the road). This impact will be partially offset by proposed habitat restoration activities (with the predicted effect varying with assumptions). In addition, the PVA shows that other mitigation activities, such as fencing on other roads to reduce mortality, or measures to increase fecundity, could compensate for the impact of the road upgrade.

Nonetheless, considerable uncertainties inevitably still exist and I recommend that a robust long-term monitoring strategy be put in place to evaluate the actual impact of the road upgrade and to inform future modelling efforts. What this monitoring strategy should look like will depend ultimately on the monitoring objectives, but the baseline studies that were used to inform the PVA surveys (e.g., Ecosure 2015) could provide an appropriate baseline for future monitoring. The outcomes of this monitoring should then explicitly inform decisions on whether to implement further mitigation activities in the future if necessary.

Yours sincerely,

Associate Professor Jonathan Rhodes