Review of Yarrabilba Koala Monitoring Program – Year 2

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Purpose of this Report

To provide an overview of conduct and outcomes of Year 2 of the on-going Yarrabilba Koala & Habitat Monitoring Program being undertaken by ***Austecology*** for Lend Lease Communities (Yarrabilba) Pty Ltd.

Context

As part of the approval of Yarrabilba Priority Development Area under the Commonwealth *Environment Protection and Biodiversity Conservation Act* as a 'Controlled Action', it is required that a Koala monitoring program be prepared and implemented as recommended in the 2012 Koala Management Plan for the site by ***Austecology.*** The relevant section of Condition 1b of the EPBC 2013/6791 Approval requires:

Development of a Koala & Habitat Monitoring Program (KHMP). A key component of developing the KHMP is the design and implementation of a 5-year koala habitat monitoring program to establish basic ecological benchmarks and to monitor initial responses of the site's koalas to development / implementation of management strategies. At conclusion, a comprehensive review will be implemented to determine successes and/or implementation of adaptive management requirements.

A KHMP was prepared and subsequently accepted by the Commonwealth and committed to by Lend Lease. The program focuses on strategies to monitor use of the site by Koalas, such as movement patterns and home range sizes, as well as other responses by the local Koala population to development activities on the site.

Objectives

In summary, the KHMP comprises a field program extending over a 3-year period – September 2017 to October 2021 and includes the implementation of three field investigation streams:

1. Bi-annual systematic surveys across the site to investigate Koala abundance and distribution.

2. The capture of Koalas for the purpose of health assessments and to tag and / or attach monitoring collars in order to assess home range, dispersal into and out of the site and habitat use. This work includes laboratory analyses of swabs taken from captured Koalas in order assess their health and genetic diversity.

3. A monthly program of fieldwork to radio-track koalas in order to make a visual assessment of Koala condition and to collect information on tree species usage.

Site Surveys

Consistent with the KHMP, the full extent of the Fauna Corridor and EPBCA Offset Areas were systematically surveyed, i.e. these constituted the priority survey footprint. Adjoining areas of green space were also surveyed to augment work within the priority survey footprint, as time permitted. There were no site access constraints which had any material impact on the success of either survey.

As directed within the KHMP, surveys were implemented twice per year, commencing in February / March and six months later in August. The survey timing in August is important, because Koala joeys are then still dependent and large enough to be detected by observers.

The following summarises the work undertaken as part of each event – survey protocols implemented were consistent with best practice guidelines and methods used within the region.

A. A site inspection was undertaken on 25 March 2019, with Koala survey work being implemented throughout the period 26 to 30 March inclusive. In August, an inspection of key survey areas was undertaken on 11 August, with Koala survey work being implemented throughout the period 12 to 16 August inclusive.
B. The average of the total survey team transect coverage for the March and August events was approximately 260 Km of foot survey transects. Each event provided a total of 15 survey person days. The survey team for both events comprised Lindsay Agnew, Brian Coulter and Heath Agnew.
C. The on-ground survey protocol provided a systematic and comprehensive search using observers working in unison, to move through habitat, following line transects and methodically searching all trees either side of the nominal centre line of their own transect for Koala presence. Visual searches for Koalas were augmented by observations of Koala faecal pellets and distinctive marks on trees.
D. The survey team was spaced approximately 50m to 60m apart either side of the nominal centre line of their own transect in order to minimize the potential for double counting from adjacent transects.

E. For any Koala observed, the following was recorded as a minimum: an assessment of the individual's condition, age and gender; GPS location of sighting; and identification of the species and DBH of the tree in which the individual was observed.

Genetic Assessment of Yarrabilba Koalas

The genetic analysis prepared (by Ms Lyndal Hulse and Dr Sean Fitzgibbon) contains valuable data and, in particular, provides a baseline against which any genetic changes that might be observed in the future can be calibrated.

This part of the program reported that 25 Koalas from the site were assessed for genetic diversity, relatedness and their population structure was evaluated based on 30 microsatellite markers – comprising 19 tissue samples from Koalas and 7 faecal pellets).

It is reported that there was an 85% match between one tissue and one scat genotype – although a 15% difference in genotype is far from conclusive that they came from the same animal, I agree with the authors that it was appropriate to exclude the data from one (the scat sample) from analysis, to preclude the possibility of bias if two samples from the same animal were to be included. I understand it is proposed that any further genetic analyses of Yarrabilba animals must utilise only tissue samples – unless and until an appropriate 'double blind' assessment is undertaken that produces compelling evidence of a very high concordance (closely approaching identity = 100%) between genotypes obtained from paired tissue and scat samples from the site.

It follows that if and when further genetic sampling of the Yarrabilba animals is undertaken, it must be based only on tissue samples and any comparisons with the current sampling and / or consolidation of the data should include recalculating the various genetic indices using the 19 tissue samples only. I reiterate the point I made in my Year 1 KHMP Overview: "Whilst some novel approaches to increase efficiency of some of these studies seem attractive, it would be very unwise to embark upon such studies without adequate site-specific validation / calibration against conventional (established) methods, e.g. standardised pair-wise references between saliva and scat samples of individuals to test the adequacy of using scat material alone as a basis for genetic assessments of the population in future."

The report summarises the genetic diversity of Koalas at Yarrabilba as showing "moderate -to-high genetic diversity, with expected heterozygosity of the population greater than 0.7". In fact, H_e for the Yarrabilba sample is in the top third of the Koala populations reported; it also needs to be borne in mind that the overall SEQ Koala population has the highest reported genetic diversity of any in Australia (and thus the world). So I presume the basis on which the Yarrabilba H_e is considered only

“moderate-to-high” is in relation to a comparison with the most genetically diverse animal populations known, especially since the Yarrabilba animals are reported as having the highest allelic richness of the populations tested? Perhaps this can be clarified in a future report. It was also concluded that there was “evidence of gene flow between populations”.

Annual Report on Koala Movements & Health

Movements

This report synthesises the findings from the four 3-day field trips spread throughout the year and includes a detailed examination of movement and home ranges for collared koalas across the entire year. These analyses are based on movement data that were collected by (a) monthly on-site radio-tracking of collared Koalas and (b) the LX remote monitoring system, which utilises GPS collars for automatic recording of the location of collared individuals twice daily.

Firstly, it is important to note that the persistent, major problem of loss of tracking collars which interfered with the program appears to have been resolved – this was a significant threat to the viability of the tracking activities.

In 2019, location data were collected for 13 koalas using LX collars or by VHF radio-tracking and showed that these individuals made extensive use of the fauna corridors as well as vegetated areas adjacent to them.

The home range analyses showed that males tended to roam more widely than females, although some females had ranges that were larger than some males. The analyses indicated that both male and female home ranges were smaller in 2019 than 2018, which the report suggests may have resulted from increased population density resulting from further vegetation clearing, but I agree it would be premature to reach that conclusion until further data are available.

The movement data showed that there were very few habitat areas in the vicinity of the collared animals that they did not utilise. Koalas are highly mobile and they can and did cross large stretches of bare ground and can make frequent use of isolated trees; though this cannot be interpreted to mean that they do not also require more intact treed areas for survival.

The accumulating movement data are essential to assess temporal variability in home ranges and movement patterns and thus provide the baseline for identifying potential responses of the site’s Koalas to further development activities on the site.

Health Assessments

During 2019, 13 independent Koalas were captured and monitored, with a relatively even sex ratio of 6 males to 7 females; as well as 2 dependent young (1M, 1F). This brought the total number of Koalas that had been examined at the site since 2017 to 20. Of the 13 independent Koalas examined in 2019, 7 had been tagged prior to 2019 – including a male which was the first Koala tagged on the site (in May 2017).

The ocular and urogenital swabs collected during the fieldtrips were tested using a quantitative polymerase chain reaction (QPCR) test, which amplifies chlamydial DNA if present on the swab samples. Whilst it may be correct that currently "this is the gold standard method of testing for chlamydial infection" it still has a detection threshold and so is unlikely to detect very low-level latent infections. Chlamydial infections in Koalas are almost always endemic NOT due to an epidemic; if tested sufficiently rigorously ALL Queensland Koalas so tested have been found to be infected. So 'PCR negative' is not synonymous with 'uninfected' or 'Chlamydia free'.

The reported health (as visually assessed) and swab test results for the 13 Koalas that were examined in 2019 provide a valuable insight into the occurrence of chlamydial infections in Yarrabilba's Koalas. The male swabbed in July (when he was QPCR positive) and again in November (when he was QPCR negative) provides an example of tolerance to the infection; this 'spontaneous resolution' demonstrates why it is contraindicated for Koalas to be removed from the site solely on the basis of a positive QPCR titre. Indeed, the majority of the animals with detectable QPCR titres were asymptomatic (showed no detectable clinical signs of chlamydiosis – i.e. 'disease'). The multiple demonstrations of poor correlation between pathogen load and clinical signs of disease (observed in other published studies) is also well demonstrated by the Yarrabilba findings, with two of the females recorded as being QPCR positive having very different chlamydial titres that did not correspond to their clinical signs.

The Koala Ecology Group report states "In 2018, nine of 10 examined koalas appeared healthy and without overt signs of disease; eight of these returned negative PCR test results for chlamydial infection (i.e. at least 20% of the population was infected with Chlamydia based on these results)." In reality, the interpretation should be that 20% of the tested animals had infections detectable using QPCR whilst the other 80% probably had undetectable infections. "In 2019, six of 12 examined koalas tested positive for Chlamydia (50% of population)" – true, but this probably does not mean there was an increase in the prevalence of infection, rather than an increase in detection. "Assuming that the examined koalas are representative of the broader population, the test results represent a significant increase in the level of detected chlamydial infection in the population, over a short period." Correct - what is demonstrated is that there was an increased prevalence of QPCR detection, which may suggest there could have been increased shedding of

chlamydial ‘elementary bodies’ (EBs) by those individuals; which is not synonymous with an increased prevalence of infection *per se*.

The report is quite correct that from the data available “It is impossible to be certain about the driver(s) that led to this increase (*in QPCR detection*)”, but given the infection is endemic, I consider it to be unlikely that the increase in QPCR detection resulted from “increased transmission between individual koalas.” The report includes some discussion of other ways in which increased QPCR detection and / or clinical signs of disease may increase due to “reduced resilience to chlamydial infection in koalas.” But “resilience” covers ‘resistance’ (i.e. the ability not to become infected when exposed to a pathogen) or ‘tolerance’ (i.e. the ability of the host’s immune system to prevent an infection from progressing from a latent / benign / asymptomatic status to a clinical disease state). It is ‘tolerance’ to chlamydial infections that is mostly the response in Koalas to variants of chlamydiae that they commonly encounter.

The report also states “In 2019, relatively few infected koalas displayed overt signs of disease” (with which I concur). The report then contends that “Treatment of such koalas generally has the best prognosis as it may halt progression of the infection to debilitating disease.”

It is unclear to me what “such koalas” refers to: if referring to the animals displaying “overt signs of disease”, then I agree; if referring to asymptomatic animals with detectable QPCR titres, then I disagree – there is no compelling evidence for this that I am aware of.

The report continues “the apparent increase in the number of koalas detected as infected with Chlamydia is cause for concern”. I agree that if there were to be an ongoing increase in the prevalence of QPCR positive individuals, this should provoke further attention, but ‘management intervention’ should not be synonymous with removal of animals from the site principally on that basis.

“In light of this evidence, we strongly recommend that an approach to managing diseased koalas that aims to reduce the overall incidence of Chlamydia in the population becomes part of the study.” Whilst a worthy objective, this would require a significant study in its own right – if there were to be just a series of *ad hoc* interventions without adequate controls, any observations will be meaningless and potentially the overall study findings will be corrupted.

“This strategy may need to include administration of antibiotics, which are commonly used in the treatment of koalas infected with Chlamydia”. I disagree – these propositions are based on misconstruing the situation.

(a) Even in tissue culture it has proven impossible to completely eliminate EBs by the use of antibiotics – so this approach almost certainly will not reduce prevalence of chlamydial infection in the Yarrabilba population.

(b) The treatment of animals with high QPCR titres might be argued to effect a lowering of the quantity of EBs being shed into the environment: but (i) it is hard to know whether 'antigenic overload' is likely to be a factor leading to latent infections progressing to disease at Yarrabilba; (ii) a practical in situ antibiotic treatment protocol (for animals other than those exhibiting severe clinical signs of chlamydiosis) would need to be devised that will achieve the goal of a significant reduction in the shedding of viable EBs; (iii) the cost / benefit for Koalas of such therapy if it involves removal into care to deliver the therapy (the additional stress of capture, transport to a care facility, the risk of cross-infection from other Koalas whilst in care and transport back to the site for release) may be justifiable for animals exhibiting significant chlamydiosis, but in my estimation is likely to be quite counterproductive for individuals not so afflicted.

(c) Unless Yarrabilba really is a closed population, the strategy is almost certainly of limited effectiveness.

I agree with the recommendation that QPCR testing of all captured koalas be continued in 2020, with repeat testing undertaken where possible. However, the assertion that "Chlamydia is a sexually-transmitted bacteria (sic), so it can spread quickly through a population (especially where there are many individuals in a relatively small area)" is rather misleading. Venereal transmission is only one of a number of modes by which Koalas can become infected. Thus, keratoconjunctivitis is unlikely to be spread venereally ('contact' is more likely) and 'vertical transmission' of chlamydiae from mothers to pouch young (via the 'pap' which is vital to inoculate the baby's gut with its essential microbiome), is highly likely. It is unclear to me what is meant by "The likelihood that treatment efforts will be successful is greatly increased if infections are detected early (i.e. before there are serious pathological consequences such as reproductive cysts and bladder wall thickening)" – this seems again to be predicated upon conflation of infection with disease. There is a valid discussion to be had about treating individuals exhibiting clinical signs of chlamydiosis before morbidity becomes irreversible, but intervention solely on the basis of a positive QPCR titre is not generally warranted.

In my overview report on Year 1 of the YKHMP I advised, "It is also important for the validity of the study as a mechanism to enhance survival and wellbeing of the Yarrabilba Koalas overall, that guidelines be agreed concerning removal of study animals from the local population." I reiterate that explicit criteria need to be agreed upon with respect to taking animals into care, including but not necessarily limited to: (i) a positive QPCR result, of itself, should not be the basis for removal of a Koala from the site; (ii) observation of clinical signs of chlamydiosis (clinical disease), on the other hand, should require a response of frequent monitoring of the individual; (iii) if an individual shows signs of substantial discomfort or incapacitation (e.g. severe bilateral keratoconjunctivitis that renders it functionally blind, or the Koala is found at the base of a tree unable or unwilling to climb, or it exhibits signs of a

serious physical injury, etc.) this should trigger immediate steps to have the animal taken into care and presented for appropriate veterinary treatment.

Conclusions

My appraisal leads me to conclude the conduct of Year 2 of the YKHMP generally meets the requirements of the relevant section of Condition 1b of the EPBC 2013/6791 Approval.

Valuable data have been acquired which provide important benchmarks in evaluating the impacts of this substantial development on the Koalas present on and proximate to the site

As I articulated in my Year 1 overview, “The main value of the YKMP is that it is longitudinal and prospective. It is, therefore, critically important that the various methodologies established at the outset are maintained consistently for the course of the Program, with any modifications carried out in a ‘double blind’ manner in parallel with original methods, so as to preserve comparability of the findings. I would be happy to provide specific advice on any proposals to ‘update’ aspects of the YKMP.”