Submission relating to the NSW koala strategy

Dr Ben Moore, Hawkesbury Institute for the Environment, Western Sydney University
b.moore@westernsydney.edu.au

I am a senior lecturer and researcher in Ecology at the Hawkesbury Institute for the Environment and the University of Western Sydney. I commenced koala research in 1997 when I started my Ph.D. at the ANU under the supervision of Bill Foley, Steve Cork and Kathrine Handasyde. I subsequently worked in north Queensland where I continued research into spatial and ecological patterns in nutritional and chemical quality of *Eucalytpus* foliage, with a focus on the ecology of the common brushtail possum. I then worked for some years overseas before returning to Australia to take up my current position and recommence koala research in 2012. I have attached a list of my publications relating to koala nutritional and chemical ecology at the end of this submission.

Current research activities of my group can be divided into six streams related to koalas. 1. The koala gastrointestinal microbiome; 2. Understanding koala diet composition; 3. Linking eucalypt chemistry to nutritional quality; 4. Understanding the effects of climate change (drought, heat and elevated CO_2) on *Eucalyptus* nutritional quality and palatability; 5. The effects of temperature on herbivores; 6. Understanding how genetics and the abiotic environment both contribute to influence eucalypt foliar quality. I will elaborate on each of these areas below and link them to the needs of the NSW koala strategy. Key personnel are Dr Michaela Blyton (postdoc) and Ms Kylie Brice (Ph.D. student).

1. **Koala gastrointestinal microbiome.** The GI microbiome refers to the micro-organisms that inhabit animals' gastrointestinal tracts. Overwhelming and rapidly accumulating evidence shows that the microbiome is an essential component of host health, nutrition and immune development and function. The microbiome plays an essential role in digestion, by helping to break down many recalcitrant components of diets, particularly starches and fibre, and this is particularly important for herbivores. Some microbiomes play key roles in detoxification of plant toxins, and work elsewhere has demonstrated that the manipulation of microbiomes can alter the ability of herbivores to tolerate particular plant toxins and consume particular foods (e.g. goats and cattle feeding on *Leucaena*, a tropical woody legume that contains the toxin mimosine, and specialist American woodrats, which must toxify oxalate in order to consume cactus foliage). I lead an ARC Linkage-funded project, with collaborators at the ANU, University of Queensland, Conservation Ecology Centre (Vic), OEH, and two universities in Denmark, that is investigating variation and the function of koala microbiomes throughout Australia.

   Already we have found that koalas from the same population at Cape Otway (Victoria), in which individual koalas feed on two different diets – the preferred *E. viminalis* and the non-preferred *E. obliqua* – have very different microbiomes. These microbiomes differ again from those of koalas from the Blue Mountains, which feed on a diverse diet and have an enormously richer and more diverse microbiome. Another part of our research shows that translocated koalas in Victoria change their microbiome after release. That microbiome composition is strongly influenced by diet was expected, but that relatively subtle differences such as those between different eucalypt species, should produce such contrasts was not.

The patterns detected so far suggest that koala microbiomes may be closely matched to the eucalypt diets consumed, and conversely, that a koala's microbiome and its species richness may constrain individual koala's diet choices. We have sampled microbiomes from 20 koala populations around Australia so will continue to expand our understanding, but the other aspect of this project relevant to the NSW koala strategy concerns the implications of the microbiome for successful rehabilitation and translocation of koalas.

We are planning a trial faecal transplant to test whether we can alter the microbiome of individual koalas and with it increase the breadth of the koala's fundamental dietary niche. If successful, these strategies may significantly improve the ability of koalas to adapt and survive after translocations (e.g. from mining or other developments, or to supplement or re-establish dwindling populations) and after release post-rehabilitation. This is particularly important in the latter context, because koalas under veterinary care very frequently receive anti-biotic treatment for *Chlamydia* or other infections) and are supplemented with nutrient-dense food formula which may catastrophically alter their microbiome, affecting the survival post-release. Many carers already attempt to inoculate rehabilitated and joey koalas with "poo shakes", but these are unlikely to be effective in the form used.

2. **Understanding diet composition.** Numerous lists of koala food trees have been compiled over the years, primarily from observations of daytime tree use. While the most significant tree species have certainly been identified in a statewide context, the use and relative importance of less common or widespread species may be over or under-estimated. Ideally, food trees should be identified on the basis of direct koala feeding data, but this is difficult to collect.

   I have used acoustic telemetry and 24-hr radiotracking observations to collect precise feeding data in the past, but this is labour-intensive (and not much fun!). Other researchers have used histological analysis of koala scats to match leaf cuticle in the faeces to candidate feeding species in the area. While this may work in some areas, in others it does not. For example, in the Blue Mountains, the cuticle of key feed species cannot be distinguished on reference slides, let alone in the faeces. From a scientific perspective, I have always found it troubling that identification of faecal cuticle of different eucalypt species can apparently not be codified in a dichotomous key for use by other researchers, because this implies more subjectivity in the assessment of slides than I think is appropriate.

   For this reason, we are attempting to develop a molecular method for the characterization of koala diet composition. Eucalypt genetics is messy, and many commonly-used barcoding genes that we have used to date, such as ITS, ETS and matK, cannot separate closely-related species. We are now attempting to use a Diversity Array Technology Sequencing (DArTseq) approach to identify markers across entire eucalypt genomes that reliably separate eucalypt species and which can be identified in koala faeces.

   Our current efforts can be described as a pilot project, focusing on only two sites, but with more funding, the diversity array that effectively forms a reference library could be readily expanded, to provide a tool for identifying koala diet composition across

NSW. This would offer a scientifically robust method for identifying what koalas are eating throughout the state and in particular regions.

I see this as essential basic biological data that is lacking for the koala (and a great many other herbivore species (DeGabriel et al. 2014), but which is key to any attempt to understand the habitat needs of a specialist herbivore, and for that matter to map koala habitat. For example, even the newly-revised SEP44 koala tree species list appears to have some omissions, based upon my own research and observations (e.g. koalas in NSW feed and rest in several ironbark species, *E. deanei* and some *Allocasuarina* species). *Syncarpia glomulifera* is also a very important rest tree and we suspect that some koalas at least occasionally fed from it.

3. **Linking eucalypt chemistry to nutritional quality.** Previously, we have demonstrated the importance of *Eucalyptus* toxins such as formylated phloroglucinol compounds (FPCs), plant antinutrients such as tannins and nitrogen (protein) to diet selection and nutrition of koalas and other eucalypt folivores (Moore and Foley 2000, 2005, Degabriel et al. 2009, Moore et al. 2010, Marsh et al. 2014). However, there is more to learn, including about the implications of eucalypt chemistry for koala health, as well as diet selection. We are currently applying our understanding of eucalypt chemistry to the study of koala ecology at Gunnedah, in a project led by Mathew Crowther (USyd) on which I am an investigator. Other researchers I collaborate with, including Kara Youngentob and Bill Foley at ANU, with PhD student Jessie Au, are investigating whether remote sensing approaches can be used to characterize the nutritional and chemical quality of eucalypt foliage from aerial imagery.

   Current research in my lab and collaboratively is investigating new FPC compounds, the fate of condensed tannins in the koala GI tract and how variation in pectin and fibre quality is linked to the functional profile of the GI microbiome and the ability of koalas to extract energy from eucalypt foliage. I am keeping a close eye on colleague's research into emerging question of the importance of foliar sodium for koala health.

4. **Understanding the effects of climate change (drought, heat and elevated CO_2) on *Eucalyptus* nutritional quality and palatability**. Notably lacking from the chief scientist's report into the Independent Review into the Decline of Koala Populations in Key Areas of NSW was any elaboration of the threat posed by climate change to koalas, beyond the direct impacts of heatwaves. In fact, climate change poses multiple potential threats to koalas, most of them indirectly via effects on the quality of their food source. While most of these threats are understood in general terms, or from other systems, much more research is required to understand whether and how eucalypts and koalas will be affected. My colleagues and I at the Hawkesbury Institute for the Environment (HIE) are leading research in this field, and are in the fortunate position of having access to remarkable experimental infrastructure and facilities.

   a. **Effects of elevated CO_2**. Because plants rely on the fixation of atmospheric carbon dioxide for the production of biomass, an increase in its concentration can produce many changes to plants' carbon economy, physiology and biochemistry. Atmospheric CO_2 concentrations are currently 403 ppm and still increasing from a preindustrial baseline of 280 ppm. The effects of elevated atmospheric CO2 levels on tree physiology (Medlyn et al. 2016) and leaf chemistry are variable and hence difficult to predict, although several general patterns have emerged. Elevated CO_2 can increase plants' water use efficiency, making them more drought-resistant, and increase growth rates. Tree foliage generally shows an increase in total phenolic and condensed tannin concentrations and decreases in terpene and nitrogen concentrations, although the latter can be ameliorated by increased temperatures (Robinson et al. 2012, Gherlenda et al. 2015). Evidence for the response of eucalypts is mostly drawn from glasshouse studies with seedings and shows a variety of responses in foliar chemistry, from no change (McKiernan et al. 2012) to increases in concentrations of some secondary metabolites and decreased foliar nitrogen under some circumstances (Lawler et al. 1997, Gleadow et al. 1998, Novriyanti et al. 2012). Eucalypts are evergreen species with long-lived leaves and their growth is often limited by phosphorus availability, and it seems that this combination of factors may limit the extent of changes to foliar chemistry, just as it has prevented increased productivity in a mature *Eucalyptus tereticornis* forest exposed to elevated CO_2 for three years at our Western Sydney University EucFACE experiment (Ellsworth et al. 2017).

If these changes are seen in koala food trees, the net result is likely to be poorer nutritional quality, as nitrogen concentrations decrease and protein becomes less digestible. Our work with brushtail possums in north Queensland (Degabriel et al. 2009) suggests that this may result in reduced breeding success, and by extrapolation, reduced population growth rates. Toxicity of leaves may also increase.

The major HIE facility for investigating the effects of elevated CO_2 on plant growth is the Eucalyptus free-air carbon dioxide enrichment (EucFACE) experiment. This experiment comprises 6 large rings (each 20 wide by 30 m high) in Cumberland Plain Woodland at Richmond. These rings fumigate the *E. tereticornis* forest to achieve an elevated CO_2 concentration, and tree growth, physiology and chemistry, are monitored at ground level and from 6 canopy cranes. This experiment will provide the first evidence about the responses of natural, adult eucalypt trees to elevated CO_2, but conclusive results have not yet emerged.

Other facilities for climate change research at HIE include whole-tree growth chambers, which allow the growth of small trees (to 9m) in a variety of atmospheric and temperature conditions. and CO2 and temperature-controlled glasshouses.

b. **Effects of drought on eucalypt leaf quality.** Because drought affects plants growth rates, it might also produce changes to leaf nutritional quality, in addition to changes in the amount of young leaf available to koalas and the water content of those leaves. At HIE, six large rainout shelters allow us to manipulate the amount of rain received by small trees, growing in competition in the ground. We are about to harvest the first major experiment from the facility, which compares the leaf quality response of 6 river red gum provenances to drought.

c. **Effects of temperature on eucalypt leaf quality.** Like the other climate changes mentioned, ambient temperature can affect the growth rates of eucalypts and the quality of foliage. HIE facilities to investigate this include whole-tree chambers, walk-in growth chambers and temperature-controlled glasshouses. Elevated temperature and elevated CO_2 can often have opposite effects on leaf nitrogen concentrations, and it is important to study these drivers simultaneously. Critically, different eucalypt species and provenances can respond differently to these drivers (Gherlenda et al. 2015)

5. **The effects of temperature on herbivores.** While the effects of high ambient temperatures on koalas during heatwaves are well-documented, less well-understood are the consequences of increased ambient temperatures for koalas' ability to detoxify plant toxins (Moore et al. 2015). At temperatures above a mammal's thermoneutral zone, liver function is reduced, effectively making toxins more toxic. This has long been understood by the pharmaceutical industry, but only now is it receiving attention in the context of wild herbivores. With Bill Foley (ANU) and Denise Dearing (Utah), I am investigating temperature-dependent toxicity in marsupials with feeding trials and direct calorimetry. If these effects are upheld in koalas, the consequence will be that as koalas experience more time at temperatures above their thermoneutral zone, their dietary choices will be narrowed, and the costs of feeding will increase, adding to other physiological and nutritional stresses associated with climate change.

6. **Understanding the influences of genetic origin and environment on eucalypt foliar quality.** Understanding within- and between-species variation in plant chemistry is a long-standing and ongoing research interest of mine (Moore et al. 2014). In my Ph.D. I demonstrated that even within a single primary food tree of the koala (tallowwood), toxin and nutrient concentrations varied dramatically across the landscape, and particularly along altitudinal gradients. This has an important implication for habitat mapping, because it means that trees of a single species should not always be treated equally wherever they occur. I revealed similar relationships and further linkages between leaf quality and soil quality in north Queensland, and I am pursuing these links further at Gunnedah with Brian Wilson (UNE and OEH).

   An area which needs more research is understanding to what extent changes in leaf quality of species across the landscape are driven by genetic differences, abiotic environmental differences or both.

<u>Relevance of my research to the NSW Koala Strategy</u>

I believe that all 6 research streams that I have outlined have direct relevance to any strategy to ensure the survival of the koala in NSW.

- Recent microbiome research has shown that understanding the role of microbes is essential to understanding the biology of any species. As a specialist herbivore with a particularly challenging diet, the link between the microbiome and the koala's wellbeing is likely particularly strong, and understanding this can help us to understand and perhaps improve the health of koalas.
  This research is relevant to the following actions from the proposed PAS:
    - *Inadequate support for fauna rehabilitation*

- *Lack of knowledge (poor understanding of sources of trauma and mortality)*

- Achieving a better understanding of what tree species koalas eat, and where, is essential to mapping koala habitat (recommendation 3) and identifying priority areas of land for management and threat mitigation (recommendation 7).
  This research is relevant to the following actions from the proposed PAS:
  - *Loss, modification and fragmentation of habitat action 1*
  - *Lack of knowledge (poor understanding of animal movements and use of habitat)*

- Koalas don't choose trees on the basis of their species, but rather on their physical and chemical attributes, and achieving a better understanding of this all contributes to our understanding and mapping of habitat quality.
  This research is relevant to the following actions from the proposed PAS:
  - *Loss, modification and fragmentation of habitat action 1*
  - *Lack of knowledge (poor understanding of animal movements and use of habitat)*

- Climate change poses numerous direct and indirect threats to koalas in NSW and may fundamentally change the calculus of which populations can be saved. The Hawkesbury Institute for the Environment has resources to investigate this which are unparalleled anywhere, but this work is expensive and recruiting students is challenging because of the dwindling number of postgraduate scholarships available.
  This research is relevant to the following actions from the proposed PAS:
  - *Human-induced climate change*

- Like the indirect effects of climate change, the potential toxicity-mediated effects of climate change on koalas was entirely ignored in the chief scientist's report, largely because research is in its infancy. Awareness of these processes needs to be increased.
  This research is relevant to the following actions from the proposed PAS:
  - *Human-induced climate change*
  - *Heat stress through drought and heatwaves*

- Understanding the roles of genetics and environment in determining foliar quality has important implications for mapping habitat quality, but also for the choice of seed stock for revegetation and creation of koala habitat. We know that aspects of leaf quality can be highly heritable (O'Reilly-Wapstra et al. 2004), so choosing the right genotypes can be important if planting unpalatable trees is to be avoided.
  This research is relevant to the following actions from the proposed PAS:
  - *Loss, modification and fragmentation of habitat (habitat planting)*
  - *Lack of knowledge (poor understanding of animal movements and use of habitat)*

Other research areas neglected in the chief scientists report and SOS project include:

- Investigating the consequences of fire for the quality of regrowth foliage. Also, even low-intensity fires can scorch canopies and the immediate significance of this for koalas is not known. What are the effects of burns of different patch sizes for koalas and what is the interval before koalas can again be supported?
    - *This should be incorporated into the PAS action relating to burning*

- Climate change is predicted to favour the growth of vines over woody trees. This may affect the quality of habitat for koalas if weedy or native vine growth impedes access of koalas to trees.

<u>Comments on the Chief Scientist’s Report:</u>

- Recommendation 1: This is a commendable approach. An example of where a whole-of-government approach has been lacking is that although I was contacted to make this submission, I was not notified either by OEH or by the Planning Department, that submissions has been called for the revised SEP44.
- Recommendation 2: Information about the distribution and abundance of koalas is essential, and attention should be paid to establishing the existence of low-density populations. In places like the Blue Mountains, occasional sightings are widespread, suggesting that low densities of animals may occur over a large area, perhaps summing to a significant population. The importance of understanding trends in koala numbers over time, particularly long time periods equivalent to ENSO cycles, cannot be overemphasized.
- Recommendation 3: I was uncertain whether “predictive habitat map” meant a habitat map for predicting where koalas are, or a map predicting where potential koala habitat is. This is an important distinction and needs to be clarified. Page 12 of the Chief scientist’s report talks about validating a map of habitat suitability for koalas against actual koala occurrence – this seems problematic because it is conflating the fundamental and observed niches of the species, despite the fact that the entire report could be read as a list of reasons why koalas do not realise their fundamental niche.
- Recommendation 8. I look forward to the symposium.
- Recommendation 9. The establishment of a single repository for koala genetic information is a worthwhile goal, but I would caution about mandatory collection of samples (as opposed to mandatory depositing of collected samples), as I have heard some researchers argue for, without further development of aims and hypotheses. Otherwise there is a risk of oversampling and sample collection for its own sake.

I hope you find this submission useful, and I look forward to further discussions at the planned symposium and elsewhere. Please do not hestitate to contact for clarification of any of my comments

Ben D. Moore

Ben Moore

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Appendix – Research by Ben Moore relevant to koala ecology and eucalypt leaf quality

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