The dietary preferences of koalas, *Phascolarctos cinereus*, in southwest Queensland

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ABSTRACT

The koala population in southwest Queensland is a large low-density population of important conservation value which is vulnerable to habitat loss, drought and climate change. The nutrient quality of *Eucalyptus* food trees favoured by koalas is an important factor influencing the survival of the koala on a low-nutrient-and-high-toxin diet. This study investigated the relationship between the diet of koalas, and food tree characteristics. Vegetation surveys, cuticle analysis and leaf chemical analysis were conducted in 14 study sites in southwest Queensland during the winter of 2010. Koala diet composition was different to eucalypt tree species availability, with *Eucalyptus camaldulensis* (56.5%) the most important tree species, *E. coolabah* (15.4%) and *E. populnea* (12.4%) of secondary preference. Leaf chemicals (moisture, total nitrogen, total phenolics, and a nutrition index = (moisture*nitrogen) / total phenolics) were significantly related to tree species, surface water availability, soil type and proximity to major creeks. Only leaf moisture was significantly correlated with koala food tree species preference. The presence of surface water appears to be a crucial characteristic of suitable koala habitat while riparian habitats dominant by *E. camaldulensis* are critical for conserving the koala populations in southwest Queensland.

Key words: conservation, diet, leaf chemistry, marsupial folivore, water content.

Introduction

Species at the margins of their geographic ranges are most vulnerable to climate change, through physiological stress and also through the decline in nutrient richness of their food sources (Ellis *et al.* 2010; Moore and DeGabriel 2010). The koala *Phascolarctos cinereus* is an arboreal marsupial folivore endemic to Australia. Its geographic range includes eastern coastal forests as well as remnant *Eucalyptus* forests in riparian areas in semi-arid eastern inland regions (Hume 1999). Koalas are recognised as specialist marsupial folivores as they show high dietary preference for *Eucalyptus* foliage and feed on a few *Eucalyptus* species within their home range (Moore and Foley 2000; Tyndale-Biscoe 2005). Compared to other non-*Eucalyptus* food sources (e.g. grasses, legumes), *Eucalyptus* foliage is low in nutrition (minerals, protein and non-structural carbohydrate) but high in indigestible or toxic materials (cellulose, lignin and plant secondary metabolites (PSMs) as a consequence of adaptation to the Australian environment (Hume 1999).

Tree use and distribution of marsupial folivores are linked to environmental conditions, such as water availability and nutrition availability, which can influence foliar chemistry and palatability of foliage for marsupial folivores (Noble 1989; Moore *et al.* 2004b). Nutrient thresholds were found to be the first restriction for the occurrence and population viability of marsupial folivores (DeGabriel *et al.* 2010). Nitrogen appeared to be more important to the koala, because levels of nitrogen in *Eucalyptus* foliage are low and barely meet the requirements of the folivores, while other nutrients are found at adequate levels (Moore *et al.* 2004a). Phenolics, a group of PSMs, act as anti-nutrition by reducing the digestibility of nutrients, especially nitrogen (Hume 2005). Although the use of the nitrogen / total phenolics (N/TP) ratio is often recommended to represent foliar nutrition, there is a negative correlation between these two foliar chemicals in *Eucalyptus* species, so N/TP may not be a better indicator than nitrogen alone in assessing foliar nutrition (Foley *et al.* 2004).

Water content in foliage was found to be related to water availability such as rainfall and free water and was essential for the survival and reproduction of koalas through extreme heat and drought in both humid and semi-arid areas (Gordon *et al.* 1988; Clifton 2010; Whisson and Carlyon 2010). In semi-arid areas, the water requirement of koalas was mostly fulfilled by foliar moisture, which was significantly related to the presence of koalas in northwestern Queensland (Munks *et al.* 1996). Thus foliar moisture should be considered in evaluating nutritional value of *Eucalyptus* leaves for koalas, especially those living in semi-arid areas.

A study in the mid-1990s estimated that there was a significant koala population living in the semi-arid Mulga Lands bioregion in southwest Queensland (Sullivan *et al.* 2004). Understanding the factors influencing the dietary preferences of this population will help assess and conserve the habitat of koalas in this region. Few studies have focused on the koala population in this region; however, there is evidence of some patterns in their distributions and diets (Witt and Pahl 1995; Sullivan *et al.* 2003a). In northern Queensland, koalas prefer habitats close to creek-lines although they use a wide range of land types (Munks *et al.* 1996). A similar pattern of the koala distribution was found in southwest Queensland as well. Almost half of the koalas live in riverine communities dominated by *E. camaldulensis* and *E. coolabah*, whereas the rest of the population occurs in adjacent *E. populnea* or *E. thozetiana* communities (Munks *et al.* 1996; Sullivan *et al.* 2003a). Diet analysis of koalas in the Mulga Lands indicated that species of riverine communities such as *E. camaldulensis*, *E. coolabah* and, to a lesser extent *E. ochrophloia*, are important food trees (Witt and Pahl 1995; Sullivan *et al.* 2003b). The role of residual ecosystems (occurring on rocky escarpments and outcrops) with *E. thozetiana* was also emphasised, while *E. populnea* was less preferred (Witt and Pahl 1995). However, the influence of foliar chemistry on the diet of koalas in the region has yet to be studied and there is little information on the relationship between koala dietary preference and *Eucalyptus* foliar nutrition in this region.

The aim of this study was to investigate the dietary choice of koala in different habitats, and assess the ability of foliar chemicals to explain this dietary variation in southwest Queensland. Foliage chemicals included water content, total nitrogen and total phenolics, and combinations of these. The central hypothesis of this study was that foliar chemistry varies between different eucalypt species, influencing koala dietary choice and tree use across habitats. The study focused on two questions: 1) What is the relationship between foliar chemistry and environmental factors including proximity to creek line, soil type, surface water availability and eucalypt tree species; and 2) How does koala diet vary with the foliar chemistry of different eucalypt tree species?

Methods

Study area and study sites

The study area was located in southwest Queensland. The majority of sites coincided with the east and north sections of the Mulga Lands bioregion, with one site occurring in the southern Mitchell Grass Downs and one occurring in the western portion of the Brigalow Belt South Bioregions (Fig. 1). The Mulga Lands are dominated by flat to undulating plains with a number of southerly flowing river systems, and has a semi-arid climate with a highly variable and summer dominant rainfall. Average annual rainfall is approximately 480 mm in the northeast, decreasing to 292 mm in the southwest. Monthly mean temperature is highest (34.2 ºC) in January and lowest (2.9 ºC) in July. The southern portion of the Mitchell Grass Downs has a similar climate, and the southwest of the Brigalow Belt South has higher annual average rainfall (570 mm) than the Mulga Lands.

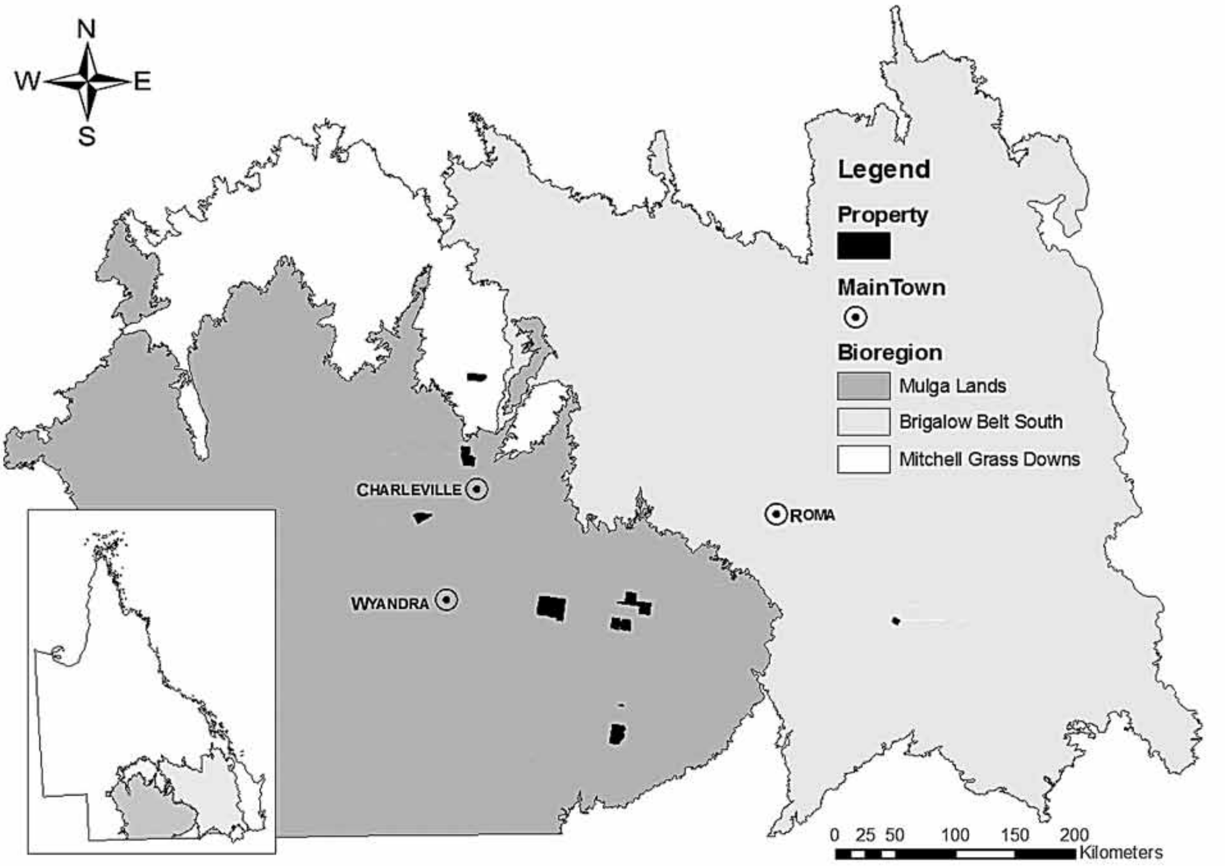

Figure 1. Study area in southwest Queensland with the location of all properties where study sites were located.

Fourteen study sites were selected from nine grazing properties based on their proximity to creeks with further refinement in the field according to vehicle access under wet conditions. The selection included a similar number on-creek (N=8) and off-creek ($\geq$ 500 m from creek lines, N=6) sites. On-and off-creek sites were characterised by different vegetation communities and soil types (Table 1). The dominant tree species in these communities (*E. camaldulensis, E. coolabah, E. populnea, E. thozetiana, E. ochrophloia*) in the study sites were reported to be used by koalas in previous studies (Witt 1993; Sullivan *et al.* 2003a). In each study site, data collected included vegetation surveys for eucalypt tree species information, koala faecal pellet collection to assess diet and eucalypt leaf sample collection for leaf chemistry assessment. The study was conducted during July and August 2010, which was a winter with above median rainfall. The region experienced a severe drought which ended in March 2010 with record floods.

Vegetation surveys

Vegetation surveys were conducted in each study site on a randomly chosen central tree and its nearest 29 trees, which was similar to that used by McAlpine *et al.* (2006) to survey koala occurrence in Noosa Shire, southeast Queensland. Only eucalypt trees with diameter at breast height (DBH) larger than 10 cm were included. Information collected at each site included site name, latitude and longitude of central tree, proximity to creek line and surface water, and soil type. For each eucalypt tree, we recorded tree species, tree height, number of stems, and DBH at 1.3 m above ground. Latitude and longitude were recorded using a global positioning system (GPS, Garmin, eTrex Legend® H). Tree height was measured using a laser range finder.

Faecal pellet surveys and leaf cuticle analysis

Leaf cuticle scale analysis of faecal pellets was used to identify the diet of koalas at each site (Ellis *et al.* 1999). For each tree, koala faecal pellet searches were conducted using a basal pellet search method described in Sullivan *et al.* (2002). Faecal pellet searches were conducted within a 1 m radius of each eucalypt tree (DBH > 10 cm) for two minutes. At least two pellets present within 10 cm of each other were assigned as one pellet-group and collected (Munks *et al.* 1996). Each pellet group was recorded as one sample and stored at ambient temperature in a brown paper bag (old and dry faecal) or in a centrifuge tube with 75% ethanol (fresh or wet faecal). In order to have similar sample size among sites, if less than two koala pellet groups were found within the site, additional pellet group(s) found adjacent to the study sites were also included. Koala faecal pellets were identified by the size, shape, smell and internal texture of pellets (Triggs 2004), and pellet age was estimated according to Table 2. Leaves of common trees species and shrubs in each location were collected and fixed in 100% ethanol as 1 cm^2 segments to make tree species reference slides.

Tree species reference slides and faecal pellet material were prepared based on the methods of previous studies (Tun 1993; Witt 1993; Ellis *et al.* 1999). Leaf segments were digested in hydrogen peroxide: glacial acetic acid

Table 1. Summary of study sites. Position of study site was defined by the distance to creek: on-creek sites were within 10 m of the creek, while off-creek sites were more than 500 m from creek.

Site	Position	Vegetation community	Soil type
1	On-creek (Water present)	*Eucalyptus camaldulensis* / *E. coolabah* / *E. populnea* / *Acacia aneura* dominant	Alluvium
2	Off-creek	*E. populnea* dominant	Red earth
3	On-creek (Water present)	*E. camaldulensis* / *E. coolabah* / *E. populnea* dominant	Alluvium
4	On-creek (Water present)	*E. camaldulensis* / *E. populnea* / *A. aneura* dominant	Alluvium
5	Off-creek	*E. populnea* dominant	Red earth
6	On-creek (Water present)	*E. camaldulensis* / *E. populnea* dominant with sparse *E. ochrophloia*	Alluvium
7	Off-creek	*E. populnea* dominant	Red earth
8	On-creek (Water present)	*E. camaldulensis* / *E. populnea* dominant	Alluvium
9	Off-creek	*E. populnea* dominant	Red earth
10	On-creek (Water present)	*E. camaldulensis* / *E. coolabah* / *E. populnea* dominant	Alluvium
11	On-creek (Dry creek bed)	*E. coolabah* dominant, with sparse *E. camaldulensis*	Grey cracking clays
12	On-creek (Dry creek bed)	*E. coolabah* dominant, with sparse *E. camaldulensis*	Grey cracking clays
13	Off-creek	*A. harpophylla* dominant with a patch of *E. populnea*	Grey cracking clays
14	Off-creek	*A. harpophylla* dominant with a patch of *E. thozetiana*	Grey cracking clays

Table 2. Criteria for the age class of koala faecal pellets (Witt and Pahl 1995).

Class	Description of class indicators
I	Fresh samples, exterior smell present, colour bright green and generally shiny. Age less than one week.
II	Lack exterior odor but retained an interior smell when crushed, dark green in colour. Age less than one month but older than one week.
III	No smell when crushed but intact and green. Age greater than one month.

(6:1 v:v) at 80 ºC in a fume hood until they turned white, indicating that the mesophyll were removed and the cuticle layers were separated. After being washed with water, the adaxial and abaxial cuticle layers of segments were peeled with forceps, the mesophyll debris was brushed off gently with a paint brush and cuticle layers were stored in 60% ethanol. They were then stained in aqueous gentian violet for one minute, washed in running water for one minute and mounted under cover slips in glycerin. Reference slides were made for six eucalypt species (*E. camaldulensis*, *E. coolabah*, *E. populnea*, *E. thozetiana*, *E. ochrophloia*, *E. melanophloia*) and examined under a light microscope at ×20 and ×40 magnification. Image taking and stomata length measurements were done using Leica Application Suite v3.3.0. Diagnostic characteristics of each species were noted and used for species identification.

A seasonal change of the tree species composition in koala diet was previously observed in the Mulga Lands (Witt 1993). As a result, only faecal pellets of age I and II were analysed to get a close approximation of the time at which the leaf samples were collected. Up to ten pellets were selected randomly from each sample and soaked in 50 ml centrifuge tubes with 5% detergent (Decon 90) for at least four days before being crushed with forceps. Tubes were centrifuged (3000 rpm, 3 min), the supernatants were poured off and the tubes filled with water. This step was repeated three times to wash out the detergent. Faecal material was bleached in 4% sodium hypochlorite solution at 60 ºC until it turned white, washed three times as before then stored in 50% ethanol. About 0.5 ml bleached material was stained with gentian violet and rinsed to remove excess stain in a laboratory sieve before mounted under cover slips in glycerin. Two slides were made for each sample as replications. One hundred fragments from each slide were identified at ×40 magnification and cross-referenced with reference slides (Sullivan *et al.* 2003b). To avoid double counting, each slide was analysed using a systematic traverse (Ellis *et al.* 1999). Only clear identifiable leaf fragments of mature leaves were included. Fragments which could not be identified were labeled as unknown species and sorted by their appearance. The mean percentage of each species identified in each sample was recorded.

Leaf chemical analysis

Eucalypt species including *E. camaldulensis, E. coolabah, E. populnea* and *E. ochrophloia* were sampled from the 14 study sites for leaf chemistry analysis. In each study site, 10 trees were randomly selected from the 30 trees of vegetation survey. Small branches in the north-east aspect of the canopy were cut down using an extendable pole with saw between 8:00am to 9:00am to minimize the impacts on leaf chemicals from sunlight. Sunrise during the survey period was around 6:50am.

The measurement of leaf moisture followed the method of Ellis *et al.* (1999). Approximately six pieces of the youngest leaves were collected from small branches in the field and weighted immediately in sealable plastic bags with an EJ-610 electronic scale (A & D Mercury) to the nearest 0.01 g. Leaf samples were stored in paper bags for air drying in the field, and were further dried in an oven at 60 ºC for three to five days in the lab (Ellis *et al.* 2002). Leaves were warm weighed daily to constant mass and the last weight was recorded as the dry weight. Leaf moisture was recorded as % wet weight.

Near infrared spectroscopy (NIRS) was used to predict the total nitrogen and total phenolics concentration of eucalypt leaves. The principle of NIRS is that when a sample is exposed to light in the near infrared spectrum, the reflected spectra can describe the chemical bonds in this sample. The spectra can be calibrated against reference values from traditional analysis of a portion of the samples. Chemical concentration can be estimated by calibration equations developed from the spectra and reference values (Foley *et al.* 1998).

About 50 g (wet mass) of young leaves were collected, air dried under shade in the field for at least two days before stored in a plastic bag and transported to the lab. Sample preparation followed the method of Wallis *et al.* (2002). The dried whole leaves were ground in a grinder (Retsch®, ZM200) to pass a 1 mm screen, and stored in an oven at 40ºC overnight to equalise sample moisture and ensure comparable moisture contents (Wallis and Foley 2003). Dry ground leaf samples were scanned by an ASD-fieldspec full range field spectrometer between 350 nm and 2500 nm with a 1.4 nm interval in the 350-1100 nm range and 2 nm interval in the 1000-2500 nm range. The spectra were measured in a dark room with quartz tungsten halogen lamp as light source. Spectra processing and modeling were done in the Unscrambler® X10.0.1. The reflectance (R) reading of each spectrum was converted to absorbance (A) values using $A = \log(1/R)$. A calibration set (N=30) was selected for laboratory analysis and modeling. The laboratory analysis of total nitrogen was done in Elementar® vario MACRO CHN, and total phenolics were analysed using folin-ciocalteau method.

Modified partial least squares regression (MPLS) was used to model the relationship between spectral characters and reference value, and full cross-validation was adopted to avoid over fitting of the model (Shenk and Westerhaus 1991). A number of combinations of scatter correction (Standard Normal Variate, Detrend or none) and mathematical treatments were applied for MPLS models to reduce the scatter effect from variable particle size and to emphasise small absorption peaks (Shenk and Westerhaus 1991; Dury *et al.* 2000). The mathematical treatments contained the order of derivative (first or second), the gap between data points used to calculate the change of spectrum, and the number of data points used to smooth the spectrum and reduce noise. A separate validation set (N=10) was selected for validation of the established MPLS models. The performance of MPLS models was assessed using the coefficients of multiple determination (R^2) and the standard error of prediction (SEP). The model with the lowest SEP and highest R^2 was selected as the best model for prediction of the remaining samples (Mark and Workman 1991).

Leaf chemical concentrations were combined as the nitrogen / total phenolics ratio (N/P) and a nutrition index (MN/P= (moisture*nitrogen) / total phenolics) to represent overall foliar nutrition value. Relationships of leaf chemical characteristics (moisture, total nitrogen, total phenolics, N/P and MN/P) to environmental factors (position of study site, water presence, tree species and soil type) were assessed using multivariate analysis of variance (MANOVA) in R version 2.14.1 (http://www.r-project.org). The relationships between leaf chemical characteristics and koala diet were tested using generalized linear regression in Statistica version 9 (http://www.statsoft.com/).

Results

Eucalypt tree vegetation of study sites

Vegetation composition and the presence of koala faecal pellets varied across study sites, properties and the proximity to creek line (Table 3). Most off-creek sites only had *E. populnea* present, while *E. camaldulensis* and *E. coolabah* dominated on-creek sites. On-creek sites had higher tree species diversity than off-creek sites. It was observed that other eucalypt species, such as *E. melanophloia* and *E. ochrophloia,* occurred as small patches or scattered trees near study sites.

Diet of koalas

Sixty-one koala faecal samples, each containing at least 10 pellets, were collected from eight study sites. Faecal samples were found in six on-creek sites but were absent in four off-creek sites. From these, twenty-three faecal samples of age I and II were selected for cuticle analysis. The cuticle remains of a feeding event are defecated between 1.5 and 6.5 days later (Sullivan *et al.* 2003b) and the faecal pellets from on-creek sites for cuticle analysis were aged less than one week in this study. Therefore, the results of this study reflected the winter diet of koalas under wet conditions. There were two to four samples from each study site as replication. On average, 97% of the cuticle fragments in faecal samples were ascribed to the six eucalypt species in the reference set. The remaining 3% of fragments were not identifiable and classified as unknown plant species according to their morphological characteristics. The proportion of tree species recorded in faecal samples differed from the availability of tree species occurring in most study sites (Table 3). According to the proportion of total fragments counted, *E. camaldulensis* (56.5%) was the food tree species most used by koalas, even when only a few trees occurred. *E. coolabah* (15.4%), *E. populnea* (12.4%), *E. thozetiana* (4.6%), *E. ochrophloia* (4.0%) and *E. melanophloia* (4.3%) were eaten in smaller proportions when available.

Leaf chemistry

Leaf moisture (% wet weight) of 140 eucalypt leaf samples had a normal distribution with a mean value of 50.06 and a standard deviation of 4.77. MPLS models were built for predictions of total nitrogen and total phenolics (Table 4). The validation showed a significant relationships between the values of laboratory analysis and those predicted by NIRS for total nitrogen (R^2 = 0.71, $F_{1,8}$ = 19.49, P = 0.0022) and total phenolics (R^2 = 0.80, $F_{1,8}$ = 32.04, P = 0.0005). These results demonstrate that the NIRS models are capable of predicting total nitrogen and total phenolics.

Table 3. Summary of the vegetation of study sites and the result of cuticle analysis, showing tree species availability versus species composition in koala diet, and koala faecal pellet-group presence. Tree species are: *E. cam= E. camaldulensis; E. cool= E. coolabah; E. pop= E. populnea; E. tho= E. thozetiana; E. mel= E. melanophloia; E. och= E. ochrophloia.*

Site	Tree availability (%) / diet composition (%)						Faecal group presence
	E. cam	*E. cool*	*E. pop*	*E. tho*	*E. mel*	*E. och*	
1	11/64.8	63/10.5	0/20.5	0/0	0/0	0/0.3	1
2	0/n.a.	0/n.a.	100/n.a.	0/n.a.	0/n.a.	0/n.a.	0
3	63/76.3	13/13.0	23/10.3	0/0	0/0	0/0	3
4	83/67.0	0/2.7	17/10.7	0/0	Observed/13.3	0/0	3
5	0/n.a.	0/n.a.	100/n.a.	0/n.a.	0/n.a.	0/n.a.	0
6	67/66.7	0/0	33/10.3	0/0	Observed/10.7	0/4.7	3
7	0/n.a.	0/n.a.	100/n.a.	0/n.a.	0/n.a.	0/n.a.	0
8	73/22.6	0/0.7	30/5.7	0/0	Observed/9.2	0/38.2	0
9	0/n.a.	0/n.a.	100/n.a.	0/n.a.	0/n.a.	0/n.a.	0
10	53/n.a.	30/n.a.	17/n.a.	0/n.a.	0/n.a.	0/n.a.	0
11	Observed/46.5	100/41.5	0/7.0	0/1.0	0/0	0/0	1
12	Observed/44.7	100/55.3	0/0	0/0	0/0	0/0	8
13	0/n.a.	0/n.a.	100/n.a.	0/n.a.	0/n.a.	0/n.a.	23
14	Observed/29.0	0/0	0/35.0	100/36.0	0/0	0/0	19

Table 4. Results of partial least squares models (N=30, with full cross-validation) and a separate validation (N=10) to the models. The R2 is the coefficient of variation between the predicted values and the laboratory values; SECV is standard error of cross-validation; SEP is standard error of prediction for validation; TN is total nitrogen (% dry matter); TP is total phenolics (mg garlic acid g-1 dry matter); Mathematical treatment contains the derivatives and the gap and data points used for smoothing functions; no scatter correction was used.

Chemical	NIRS equation performance						
	Mean	Range	Bias	R2	SECV	SEP	Math. treatment
TN	1.47	1.11-1.93	-0.00046	0.98	0.14	0.096	1,8,8
TP	25.76	5.99-57.04	-0.00014	0.99	9.71	7.655	1,4,8

There were highly significant relationships between three leaf chemicals (moisture, total nitrogen and total phenolics) and the position of study sites in relation to the creek, the presence of water, tree species and soil type (Table 5).

From means and standard errors of these significant relationships (Table 6), we can conclude that: 1) all three leaf chemicals were significantly higher with surface water presence or in alluvium; 2) leaf moisture and total phenolics were significantly higher in on-creek study sites; 3) the leaf moisture and total phenolics of *E. camaldulensis* were the highest compared to other species, while *E. thozetiana* had the lowest moisture content and *E. populnea* was of the lowest phenolics concentration; 4) but N/P and MN/P which combined three leaf chemicals were significantly lower in on-creek sites, on alluvium and with surface water present. Besides, total nitrogen of *E. populnea* (1.51±0.01) and *E. camaldulensis* (1.49±0.02) were higher than *E. coolabah* (1.38±0.02) and *E. thozetiana* (1.38±0.04).

Normal linear model with log link showed that tree species composition in koala diet had no significant relationship with tree species' average total nitrogen, total phenolics N/P and MN/P, but was significantly related to the leaf moisture (Table 7). The leaf moisture of the four tree species resembled the order of koala diet composition (Fig. 2).

Table 5. Results of multivariate analysis of variance (MANOVA) showing the relationships between eucalypt leaf chemicals and explanatory environmental factors. N/P= Nitrogen / Phenolics. MN/P= (Moisture*Nitrogen) / Phenolics. Significant codes: P < 0 '***'; P < 0.001 '**'; P < 0.01 '*'; P < 0.05 '.'; P < 0.1 ' '.

Leaf Chemicals	F value of Environmental Factors				
	Site	Position	Soil	Species	Water
Moisture	24.499 ***	28.748 ***	30.024 ***	10.985**	25.893 ***
Phenolics	1.654	33.126***	4.149 *	10.857**	9.893 **
Nitrogen	0.001	1.330	10.250**	0.817	14.796 ***
N/P	0.091	26.883 ***	1.477	1.059	10.385 **
MN/P	0.145	17.227***	0.131	0.661	13.158 ***
Critical value (α=0.05)	F(13,119)= 1.83	F(1,119)= 3.92	F(2,119)= 3.07	F(3,119)= 2.68	F(1,119) = 3.92

Table 6. Means and standard errors of significant relationships between eucalypt leaf chemicals and explanatory environmental factors. Tree species: *E. cam= E. camaldulensis; E. cool= E. coolabah; E. pop= E. populnea; E. thoz= E. thozetiana.* n.s. = not significant. N/P= Nitrogen / Phenolics. MN/P= (Moisture*Nitrogen) / Phenolics. Significant codes: P < 0 '***'; P < 0.001 '**'; P < 0.01 '*'; P < 0.05 '.'; P < 0.1 ' '.

Environmental Factors		Leaf Chemicals				
		Moisture (% wet weight)	Phenolics (mg garlic acid g-1 dry matter)	Nitrogen (% dry matter)	N/P	MN/P
Water	Present	53.28 ±0.59***	29.12 ±1.40**	1.512 ±0.01***	0.059 ±0.002**	3.13 ±0.16***
	Absent	47.64 ±0.36***	23.23 ±0.96**	1.446 ±0.01***	0.074 ±0.059**	3.59 ±0.22***
Position	On	51.44 ±0.61***	29.35 ±1.13***	n.s.	0.056 ±0.002***	2.91 ±0.13***
	Off	48.21 ±0.35***	20.95 ±0.99***	n.s.	0.083 ±0.005***	4.06 ±0.27***
Soil	Alluvium	53.32 ±0.60***	29.38 ±1.40*	1.50 ±0.01**	n.s.	n.s.
	Red Earth	48.35 ±0.37***	18.82 ±0.94*	1.49 ±0.02**	n.s.	n.s.
	Grey Clay	47.00 ±0.60***	27.52 ±1.38*	1.40 ±0.02**	n.s.	n.s.
Species	E. cam	53.21 ±0.82**	35.34 ±1.90**	n.s.	n.s.	n.s.
	E. cool	49.42 ±1.15**	26.68 ±1.50**	n.s.	n.s.	n.s.
	E. pop	49.59 ±0.39**	20.13 ±0.73**	n.s.	n.s.	n.s.
	E. thoz	45.10 ±0.54**	30.52 ±2.91**	n.s.	n.s.	n.s.

Table 7. Parameter estimates of generalized linear models (normal linear model with log link) of koala tree species diet to eucalypt leaf chemicals of tree species. N/P= Nitrogen / Phenolics. MN/P= (Moisture*Nitrogen) / Phenolics. Significant code: P < 0.05 '*'.

		Estimate	Standard Error	Wald Statistics	p
Moisture*	Intercept	-13.656	1.488	84.211	0.00*
	Moisture	0.330	0.028	137.099	0.00*
Phenolics	Intercept	-4.716	3.824	1.520	0.217
	Phenolics	0.244	0.109	4.959	0.025*
Nitrogen	Intercept	-4.831	10.340	0.218	0.640
	Nitrogen	5.443	6.975	0.608	0.435
N/P	Intercept	31.427	25.371	1.534	0.215
	N/P	-594.136	548.256	1.174	0.278
MN/P	Intercept	3.910	1.847	4.481	0.034*
	MN/P	-0.285	0.664	0.184	0.667

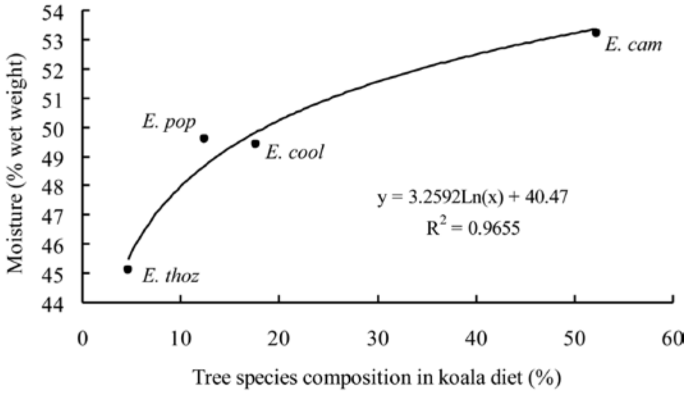

Figure 2. The relationship of tree species composition in koala diet and mean leaf moisture with trend line (solid line). Tree species: *E. cam= E. camaldulensis; E. cool= E. coolabah; E. pop= E. populnea; E. thoz= E. thozetiana.*

Discussion

Koala diet and leaf chemistry

Our study represents the winter diet of the koala, and under above-average rainfall conditions. However, results agree with previous work, finding that eucalypt species comprise at least 97% of koala diet and *E. camaldulensis* was the most preferred food species in riparian habitats, followed by *E. coolabah*, *E. populnea*, *E. ochrophloia* and *E. melanophloia*. The selection of these tree species is consistent with the five primary food tree species suggested by Sullivan *et al.* (2003b).

It is broadly agreed that only a few tree species are the primary food of koalas at any locality and the composition of preferred tree species varies from place to place (Pahl *et al.* 1984; Phillips and Callaghan 2000). In Queensland, *E. tereticornis*, *E. camaldulensis*, *E. crebra* and *E. populnea* are reported as dominant in the diet of western koala populations (Phillips 1990; Ellis *et al.* 2002; Sullivan *et al.* 2003b; Tucker *et al.* 2007). Two previous studies have conducted leaf cuticle analysis to identify the primary food tree species of the annual koala diet in semi-arid southwest Queensland. In a study focusing on the whole Mulga Lands, Sullivan *et al.* (2003b) found that 99.6% leaf cuticle fragments were from eucalypt species, including *Corymbia* species. Five *Eucalyptus* species (*E. camaldulensis*, *E. thozetiana*, *E. coolabah*, *E. populnea*, *E. melanophloia*) were identified as primary food tree species for koalas in the region, making up of 93% of the total fragments counted (Sullivan *et al.* 2003b). These five species were assigned to three groups according to the landform in which the tree species was dominant in koala diet. The most frequently detected species in all faecal pellets was *E. camaldulensis*, which was also the most preferred food tree in riverine habitat (Sullivan *et al.* 2003a). A study conducted on one property in the northern Mulga Lands emphasised *E. coolabah* was the most important food tree species in that area, followed by *E. thozetiana* in woodlands on rocky residuals, while *E. populnea* woodlands was less preferred (Witt and Pahl 1995). However, *E. populnea* and *E. coolabah* made up similar proportions of the koalas' diet in this study. This could be explained by the dietary variation due to different seasons of diet sampling or according to different vegetation compositions across the region (Witt 1993).

While previous studies in the region have identified tree species use by koalas in southwest Queensland, no studies have assessed the relationship between the diet of koalas and leaf chemistry in the region. Tree species composition in koala diet was not consistent with tree availability in sites but had a significant positive relationship with species' foliar moisture content. Neither the nitrogen / total phenolics ratio nor the moisture*nitrogen / total phenolics ratio can explain the food tree species preference of koalas. Similarly, the nitrogen-to-sideroxylonal ratio did not explain the feeding decisions of brushtail possum, another marsupial folivore (Wallis *et al.* 2002). Hence, the diet of marsupial folivores cannot be fully explained by a simple ratio (Moore *et al.* 2004a; DeGabriel *et al.* 2010), which might be caused by the complicated digestion process with chemicals reactions and bacterial impacts.

The impact of leaf moisture was significant in this study and it appeared that koalas select tree species with higher leaf moisture as primary food source in southwest Queensland. It should be noticed that this study was conducted during winter under an above average rainfall conditions with relatively low water demand of koalas and overall wet environment. Hence the relationship between foliar moisture and food tree preference may be different or even accentuated under extreme heat or drought (Clifton 2010; Ellis *et al.* 2010).

Impacts of water availability on koalas in southwest Queensland

Water availability varies both spatially and temporally which influences leaf moisture in different ways. In sub-humid central Queensland, annual records of water content in *Eucalyptus* foliage showed a trend that water concentrations were higher during summer, the rainy season (Ellis *et al.* 1995). This study found that leaf moisture was significantly higher with the presence of surface water regardless of tree species. Foliar water fulfils most of moisture requirement of koalas in semi-arid areas (Munks *et al.* 1996). With the presence of koalas significantly related to water availability and leaf moisture of tree species instead of leaf nutrition, Munks *et al.* (1996) suggested that water availability, rather than soil type, was the primary factor identifying optimum koala habitat in arid and semi-arid woodlands. This finding was supported by this study. Although there was a significant relationship between leaf moisture and soil types, the three soil types can be divided into two groups: alluvium with surface water presence; red earths and grey cracking clays without surface water present. Hence this relationship could be ascribed to water availability in sites. The impacts of water availability are not just restricted to leaf moisture but also involve foliar concentrations of PSMs by influencing growth rate of trees (Cork *et al.* 1990; Munks *et al.* 1996). There is increasing agreement on the important role of PSMs in explaining koala food choice (Lawler *et al.* 2000). It appears, therefore, that water availability is an essential determinant of leaf chemicals, and hence the food quality for koalas in southwest Queensland (Whisson and Carlyon 2010).

Food availability and quality have been found to be important in determining the viability of koala populations (Gordon *et al.* 1988; McAlpine *et al.* 2008), so habitats with more favourite food trees would be preferred by koalas and used more frequently. Results of this study suggest that habitats with higher water availability, mainly riparian habitat, provide higher food availability and quality for koalas, which agrees with the findings of previous studies. In the Mulga Lands bioregion of southwest Queensland, koalas were observed to occur in two types of habitat: 47.6% in the riparian woodlands dominant by *E. camaldulensis* and 28.6% in the residual woodlands with *E. thozetiana* (Sullivan *et al.* 2003a). Witt and Pahl (1995) reported that the koala is distributed continuously along riparian habitat and spread into adjacent favored habitats. This was supported by the results of koala faecal pellet records of recent studies by Seabrook *et al.* (2011) and this study. Faecal pellet counts in northern Queensland were highest in the creek beds and lowest beyond 100 m from creek beds (Munks *et al.* 1996). In our study, faecal pellets were present in on-creek sites and absent in off-creek sites. Therefore, although it was concluded that koalas can use different habitats seasonally and have a large foraging area based on faecal pellet age and cuticle analysis (Witt and Pahl 1995; Munks *et al.* 1996), riparian woodlands dominated by *E. camaldulensis* appeared to be the most important habitat for koalas in the Mulga Lands. Moreover, the essential role of riparian woodlands in supporting the koala population becomes remarkable as they cover only 0.9% coverage in the region (Sullivan *et al.* 2004). Therefore, results of this study support the central hypothesis that foliar chemistry varies between different eucalypt species, influencing koala dietary choice and tree use across habitats.

Limitations and future research

In this study, there was a limitation of using NIRS with dried leaf spectra for foliar chemistry assessment in remote areas. Calibration models developed from oven dried leaf samples were proved to be less precise compare to freeze dried leaves (Dury *et al.* 2000). Despite this, we did adopt this method because of the difficulty in freezing large amount of leaf samples for several weeks in a remote area. Previous studies proved that it was feasible to use fresh leaf spectra for NIRS (Dury *et al.* 2000; Ebbers *et al.* 2002). Therefore, it is worth using high spectral resolution remote sensing techniques as a tool for assessing field eucalypt foliar chemistry and mapping koala habitat quality in remote areas (Scarth *et al.* 2001; Moore *et al.* 2010).

Three components of leaf chemistry analyzed in this study were: water content, total nitrogen and total phenolics; however, the impact of foliar chemistry on food choice for marsupial folivores is more complicated than this. There has been increasing evidences of the resistance of marsupial folivores against high formylated phloroglucinol compounds (FPCs) diet, making FPCs the key foliage chemicals in explaining dietary variation of koalas and other marsupial folivores (Lawler *et al.* 1998; Marsh *et al.* 2003; Moore *et al.* 2005; Marsh *et al.* 2007). Also in vitro digestible nitrogen is a more precise indicator of protein availability than total nitrogen or the simple T/P index (DeGabriel *et al.* 2010; Moore *et al.* 2010). As a result, these chemicals should be included in future studies as more promising indicators of foliar palatability.

This study was a short term study which only reflected the folivore-foliage interaction during a wet winter. In order to fully explore the koala dietary ecology in southwest Queensland, it should be extended to summer and under dryer conditions. It would also be useful to estimate the ecological plasticity of riparian habitat under predicted climate change and assess the ability of such habitat in helping koalas to survive from severe heat and droughts (Ellis *et al.* 2010).

Implications for Conservation

Koalas were identified as one of the climate change flagship species because of the degradation of food availability and quality caused by increasing atmospheric CO_2 and extreme weather (e.g. heat-wave and severe drought) (Moore and DeGabriel 2010). It is essential to protect this species from climate change, and habitats with abundant preferred food trees are a key factor in conserving this species. The conservation value of koala populations in southwest Queensland is increasing because of its large population size and the lower conservation cost compare to that of the coastal populations threatened by rapid urbanisation (Sullivan *et al.* 2004). However, the populations are threatened by drought, rapid habitat clearance and habitat degradation by grazing (Sullivan *et al.* 2004; Seabrook *et al.* 2011). The results of this study suggest that riparian habitat is essential in maintaining the viability of the koala populations in southwest Queensland. Hence, koala conservation in this region should give priority in protecting riparian habitat because of its high conservation value and low spatial extent. Riparian habitats are protected under the Queensland Vegetation Management Act 1999. Based on the findings of this study, three key conservation implications are highlighted:

1. Habitat regeneration and habitat quality monitoring should be conducted to increase and maintain the continuity of riparian habitat and its ecological functions including serving as primary koala habitat, as corridors connecting koala populations across the region, and as refuge against severe climatic events.
2. Conservation and management of lower quality habitats such as residual habitats surrounding *E. populnea* woodlands, and other surface water bodies such as farm dams are essential for maintaining a sustainable koala population.
3. Given the key role of water availability and its impact on koala food quality, catchment management strategies should strive to prevent the degradation of watercourses due to over grazing and clearing of catchment headwaters.

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