ACT Government

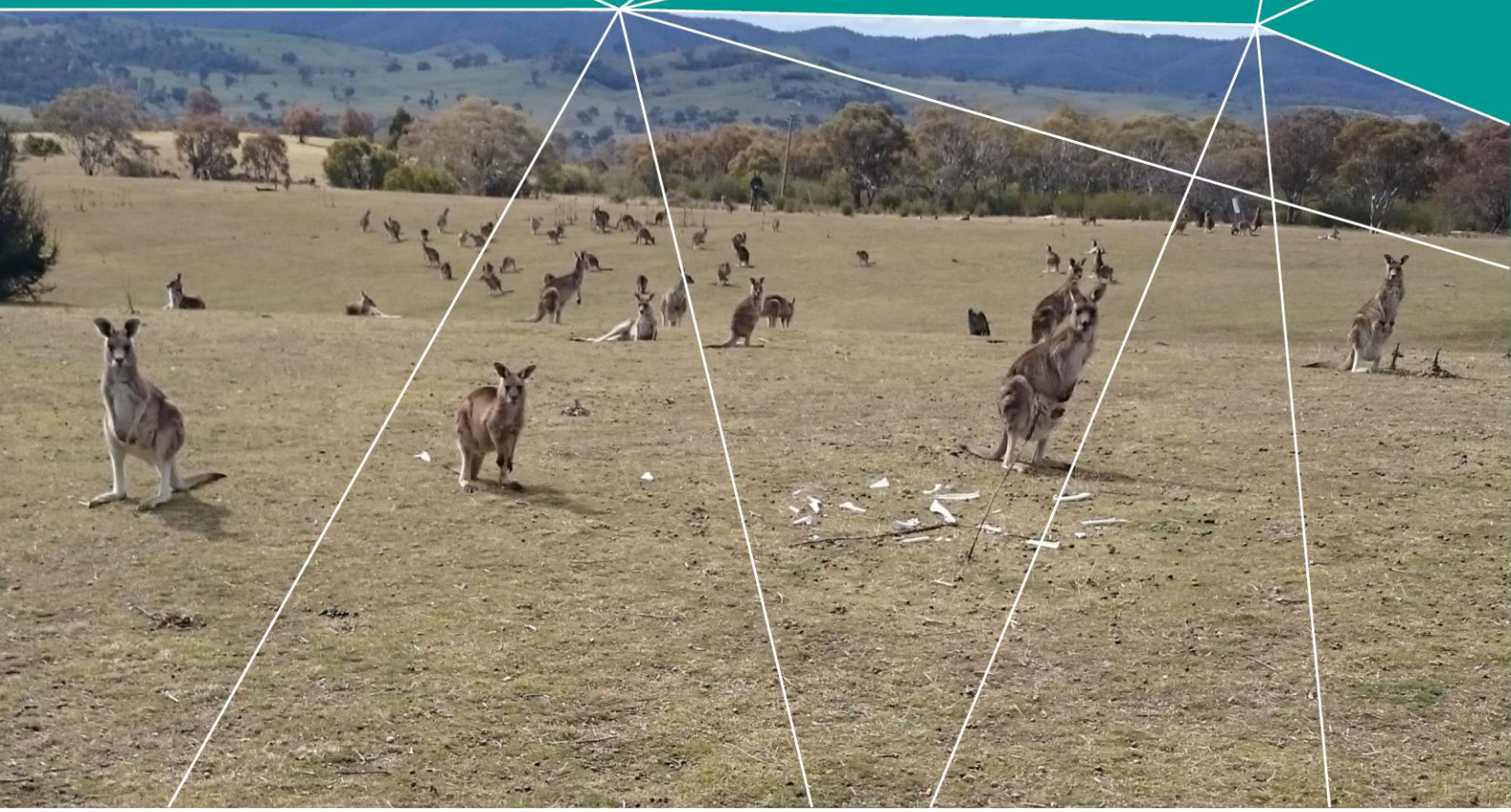
FACTORS INFLUENCING SUB-ADULT MORTALITY EVENTS IN EASTERN GREY KANGAROOS (*MACROPUS GIGANTEUS*) IN THE ACT

FEBRUARY 2018

Tim Portas and Melissa Snape

Conservation Research

Technical Report



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Front cover: High kangaroo grazing pressure maintains a short 'marsupial lawn' in the Tidbinbilla valley, revealing the bones of deceased conspecifics (M. A. Snape).

Technical Report

Factors influencing sub-adult mortality events in Eastern Grey Kangaroos (*Macropus giganteus*) in the ACT

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GLOSSARY

Albumin: a serum protein formed by the liver and the major blood protein responsible for regulating osmotic pressure of the blood

Blood urea nitrogen (urea): the main nitrogenous end product of protein metabolism

Creatinine: a metabolite of creatine phosphate (a compound that acts as a source of energy in muscle and which varies with muscle mass) which is excreted in the urine

Duodenum: the first portion of the small intestine

Globocephaloides trifidospicularis: a blood feeding nematode found in the duodenum of kangaroos

Globulins: serum proteins (including enzymes, antibodies and lipoproteins) other than albumin

Haematocrit (Hct): the volume percentage of red blood cells in whole blood

Haemoglobin (Hb): a protein found in red blood cells responsible for transporting oxygen in the blood

Jejunum: the middle portion of the small intestine

Mesenteric fat: fat found in the membranous sheet (the mesentery) that attaches various organs to the body wall

Peri-renal fat: fat found adjacent to the kidneys

Pylorus: the portion of the stomach that connects with and opens into the small intestine

Red cell count (RCC): the number of red blood cells in a given sample of blood

Total protein: a measure of the total protein in the blood (both globulin and albumin)

Triglycerides: the main lipid component of dietary fat and fat depots of animals

EXECUTIVE SUMMARY

The density of unmanaged Eastern Grey Kangaroo populations in the ACT and other temperate regions of south-eastern Australia is largely limited by the combined influences of predation (predominantly by the Dingo, Red Fox, Wedge-tailed Eagle, and motor vehicle) and the finite availability of food (predominantly grasses). The relative influences of these two major mechanisms of population density varies with predator abundance and climatic-dependent grass availability. Where populations are found to be food-limited, die-off events characterised by the presence of intact, predominantly sub-adult kangaroo carcasses, may be observed at various scales.

In the ACT, such die-off events are most commonly observed toward the end of winter and early spring, when grass availability is low due to a period of minimal growth during the cooler months, and when low overnight temperatures (as low as -8°C) may cause additional stress to kangaroo populations. Under such conditions, the carcasses of sub-adult kangaroos of approximately 18-24 months of age have been recorded in moderate numbers within various high kangaroo density nature reserves. Animals are generally found to have minimal body fat but negligible other clinical signs indicative of cause of death.

In response to the collection of port-mortem samples from such a die-off event at Tidbinbilla Nature Reserve in 2014, the current study was undertaken to more closely examine the factors underpinning mortality events in sub-adult Eastern Grey Kangaroos. Whilst indices of condition (such as body fat levels) are generally low in animals of this age relative to adults in the population, regardless of food availability, results from this study indicate that the condition of animals at high population density (and low food availability) is generally poorer than those where population density is low and food availability is not limited.

The results from this study are based on sampling conducted across four sites in the winter and early spring of 2017. Given that no reports of die-off events were made at any of these sites during the study period (indicating that conditions may not have been as dire as in previous years), repeat survey is likely warranted in future years if a full appreciation of the relative influences of population density, food availability, parasite burden and climatic factors on die-off events is sought.

BACKGROUND

Populations of Eastern Grey Kangaroos (*Macropus giganteus*) in temperate regions are considered to be limited by the starvation of a variable proportion of animals during winter and early spring each year, when *per capita* food availability is generally at its lowest (Fletcher 2006). Starvation tends to affect predominantly sub-adult (and to a lesser extent aged) animals, although in severe food shortages animals from other age groups may also be affected. The sub-adult age class is worst affected due to high energy requirements for growth and low gastrointestinal tract capacity relative to body size (Munn and Dawson 2006). Clinical signs of starvation include weakness, poor body and pelage condition with animals becoming moribund in advanced cases. Starvation is considered the ultimate cause of mortality of sub-adult Eastern Grey Kangaroos during late winter and spring, while hypothermia, endoparasitism and predation are likely proximate causes. Periodic higher than normal late winter/early spring mortality in sub-adult Eastern Grey Kangaroos has been reported, although accounts are anecdotal and limited investigation of these events has been undertaken.

From July through to early September 2015 increased mortality of free-ranging Eastern Grey Kangaroos was reported from a number of sites in the Australian Capital Territory including Tidbinbilla Nature Reserve. At Tidbinbilla Nature Reserve, where an estimated 200 kangaroos died, 10 animals (three males, seven females) were examined; one frozen-thawed carcass, four moribund kangaroos and five sick but ambulatory kangaroos. Eight of the 10 kangaroos were sub-adults (weight range 6.1-9.9 kg). Affected kangaroos were emaciated and weak but exhibited no other clinical signs. Blood collected for haematology and biochemistry (n=8) revealed anaemia (n=7), hypoalbuminaemia (n=7), mild azotaemia (n=5) and moderately elevated creatine kinase (n=4). Pertinent histopathological findings included marked gastric mucosal ulceration and enteric coccidiosis in some animals. All kangaroos harboured gastrointestinal parasites although species present and intensity of infection varied between animals. Five of seven kangaroos were PCR positive for a novel trypanosome genetically distinct from but most closely related to *Trypanosoma copemani*. Four of these kangaroos were also PCR positive for a novel *Theileria* species closely related to *T. penicillata*. *Cryptosporidium macropodum* was detected via PCR in faecal samples examined from four of six kangaroos. While endoparasitism and hypothermia, associated with below average minimum overnight temperatures, were considered contributory, starvation associated with low pasture biomass was considered the ultimate cause of death.

These findings coupled with anecdotal evidence that similar mortalities did not occur at sites with lower kangaroo population densities suggest that the nutritional status and consequently health of Eastern Grey Kangaroos at higher population densities (i.e. lower *per capita* food availability) may be compromised compared with lower population density sites. This study aimed to assess the relationships between kangaroo population density, forage availability and health parameters (haematology, serum biochemistry, endoparasite burdens, femur and bone marrow fat and body weight) of sub-adult Eastern Grey Kangaroos.

METHODS

STUDY SITES

Kangaroos were collected from four separate sites in the ACT. Sites were chosen on the basis of known historical kangaroo population densities to represent two high density kangaroo populations and two low density kangaroo populations.

TIDBINBILLA NATURE RESERVE

Tidbinbilla Nature Reserve (TNR) is a 5450 hectare reserve at an elevation of 700 - 1580 metres above sea level and mean annual rainfall of approximately 946 mm. A 274 ha open grassy area in the lower elevation region of the reserve was chosen as the study site for this investigation. The principal vegetation type in the study site consists of grassland representing a mix of exotic grasses and remnant native pasture.

LITTLE MULLIGANS FLAT

Little Mulligans Flat (LMF) is a 131 hectare reserve at an elevation of 650 – 700 metres above sea level and mean annual rainfall of approximately 650 mm. The principal vegetation type consists of box-gum grassy eucalypt woodland.

GOOROOYAROO WOODLAND RESERVE

Goorooyaroo Nature Reserve (GOO) is a 702 hectare reserve at an elevation of 650 – 700 metres above sea level and mean annual rainfall of approximately 650 mm. The principal vegetation type consists of box-gum grassy eucalypt woodland.

GUNGADERRA GRASSLAND NATURE RESERVE

Gungaderra Nature Reserve (GUN) is a 342 hectare reserve at an elevation of 600 – 650 metres above sea level and mean annual rainfall of approximately 650 mm. The principal vegetation type consists of natural temperate grassland dominated by *Austrostipa* spp. and *Themeda triandra*.

ASSESSMENTS OF KANGAROO DENSITY

Kangaroo density was assessed either using walked line transect counts undertaken according to the 'distance sampling' technique (Buckland et al., 2007; Buckland et al., 2001; Thomas et al., 2010) or a 'sweep count' modified from Coulson and Raines (1985). Specific techniques used in the ACT have been described previously (ACT Government, 2010). Briefly for distance sampling, approximately 44 km of transects are surveyed per site, based on achieving a coefficient of variation < 15% at 'average' densities for the ACT (Buckland et al., 2007). Transects are parallel, orientated NW-SE to avoid visibility being impaired by the winter morning sun, and no two transects surveyed on one day are within 600 m. Surveys aim to detect kangaroos grazing in the open during the early morning, and hence are undertaken from first light and cease either 3 hours post sunrise or when animals are observed to be laying down. The distance and bearing to kangaroo groups are recorded from the

observers position on the transect line, recorded using a laser rangefinder (TruPulse 360) and GPS (Garmin 62S). This technique enables the GPS position of kangaroo groups to be related to canopy cover strata, allowing multiple covariate distance sampling analysis to describe different 'detection functions' for kangaroo groups depending on the vegetation type in which they are observed (Thomas et al., 2010). The best fitting model for each reserve was chosen based on AIC, or model averaging where Δ AIC < 2.

ASSESSMENT OF HERBAGE MASS

Herbage mass measurements (to determine the per capita food availability at the different sites) were taken at 30 quadrats within each site during the first week of July, August and September. Quadrats were arranged in a grid pattern such that they were likely to be representative of the variety of vegetation types present across the site. Herbage mass assessments involved measurement of average grass height, a plate meter reading (Jenquip, New Zealand) and visual estimation of the percentage of vegetative cover, the percentage of bare ground and the percentage of grass which was green within 0.25 m^2 circular quadrats. The genus of the dominant grass within each quadrat was also recorded to assist with estimates of pasture palatability and the calculation of herbage mass from average height and plate meter measurements.

ANIMALS AND SAMPLE COLLECTION

Sample collection was conducted between August and October 2017. Free-ranging Eastern Grey Kangaroos at TNR and LMF were anaesthetised with a proprietary mixture of zolazepam/tiletamine (Zoletil ,Virbac Australia) administered at 5 mg/kg in combination with medetomidine hydrochloride (Ilium Medetomidine, Troy Laboratories) at 0.02 mg/kg administered intramuscularly via projectile syringe. Once recumbent, blood was collected from the recurrent tarsal or jugular vein into plain serum or EDTA tubes. Kangaroos were subsequently transported to a central processing facility and euthanased with an overdose of intravenous barbiturates. Kangaroos at GOO and GUN were killed by shooting. Kangaroos were killed by a head shot at night time using a .223 Calibre rifle with 55gr ballistic tip ammunition before being transported to a central processing facility. The sub-adult age class was targeted and where possible the sex of the animal was determined prior to euthanasia/shooting with the aim of collecting female animals where possible.

Kangaroos were weighed (to the nearest 250 grams) using a hanging scale and the following morphometric measurements (to the nearest mm) were collected; head length (where intact), forearm length, pes length, hind limb length and tail circumference. The kangaroo was then placed in right lateral recumbency and the carcass opened by removing the left lateral abdominal and thoracic walls.

Both kidneys and any peri-renal fat were dissected out and weighed to the nearest 0.01 gram using an electronic scale. Mesenteric fat was subjectively assessed on a scale of 1 to 4 with 1 being no fat present and 4 being abundant fat present. The left femur was dissected free from the surrounding muscle and following the method described by Caughley and Sinclair (1994) and Fletcher (2007), the middle third of femur was collected and the bone marrow removed into a glass jar and weighed to the nearest gram, oven dried at 70°C for 48 hours, and weighed again subsequently to determine dry

weight. The percentage of marrow fat was calculated from the wet and dry weights, with a correction factor applied to allow for the non-fat, non-water components of the marrow (Fletcher 2007):

Percentage marrow fat = (100 Dry Weight / Wet Weight) - 10

A section of gastrointestinal tract extending from the pylorus to the jejunum was removed, dissected free from its mesenteric attachments and opened along its length. The total number of *Globocephaloides trifidospicularis* present in the duodenum (and adjacent pylorus) were counted and recorded.

Animal age was estimated based on the average of ages predicted by the head length, leg length (as per Poole et al., 1982) and molar progression index (Kirkpatrick 1964, 1965). This allowed for some redundancy where some measurements were unavailable due to limitations imposed by the sampling methods used (e.g. destroyed skills, or rigour mortis).

Haematologic and biochemical analyses were performed within 24 hours of collection by Vetnostics, North Ryde, Australia. Haematologic analysis was performed on EDTA whole blood with initial processing on a Sysmex XT-2000i Automated Hematology Analyzer (Sysmex America Inc. Illinois, USA) followed by manual differential. Haematocrit, haemoglobin and red cell count were measured. Serum biochemistry was performed on the Roche Modular EVO Analyzer (Roche Products Pty Ltd, Castle Hill, Australia). Blood urea nitrogen (urea), creatinine, total protein, globulins, albumin and triglycerides were measured.

This study was undertaken in accordance with animal ethics approval (University of Canberra AEC 17-06) and the appropriate ACT scientific licence (LK20174).

STATISTICAL ANALYSIS

All statistical analyses were performed using Program R (R Core Development Team, 2008). One-way analysis of variance (ANOVA) was used to assess the overall effects of site (as a proxy for per capita food availability) and pairwise t-tests were performed to ascertain between-site differences in health parameters where an overall effect of site was found to be significant ($p < 0.05$). The effect of sex on health parameters was also assessed at GOO and GUN using ANOVA. Relationships between continuous data relating to health variables and/or morphometric measures were assessed using linear models. All relationships are presented as significant when $p < 0.05$. Results are presented as means ± standard error unless specified otherwise.

RESULTS

KANGAROO DENSITY AND PER CAPITA FOOD AVAILABILITY

Kangaroo density was higher at TNR and LMF compared to GOO and GUN as expected. Per capita green herbage mass was lower at sites with higher kangaroo density. Kangaroo densities and per capita green herbage mass for each site are presented in Table 1.

HERBAGE MASS

Herbage mass for each site is detailed in Table 1. Herbage mass was higher at GUN than any other reserve ($p < 0.001$). Herbage mass at GOO was higher than that at LMF ($p = 0.002$) but did not differ significantly from that at TNR ($p > 0.1$). When assessing green herbage mass as an index of the amount of quality food available at each site, a similar trend was observed with GUN having more than GOO ($p = 0.008$), LMF ($p < 0.001$) and TNR ($p = 0.002$). The estimated amount of green herbage mass at GOO, LMF and TNR did not differ significantly ($p > 0.2$).

Table 1. Kangaroo densities (mean and 95% confidence intervals) and both total and green herbage mass (mean ± SE) at Tidbinbilla Nature Reserve (TNR), Little Mulligans Flat (LMF), Goorooyaroo Woodland Reserve (GOO) and Gungaderra Grassland Nature Reserve (GUN).

Location	Kangaroo density (kangaroos/ha)	Total herbage mass (kg/ha)	Green herbage mass (kg/ha)	Per capita green herbage mass (kg/roo/ha)
TNR	2.39 (1.79 – 3.19)	363.6 ± 68.3	137.9 ± 26.5	57.7
LMF	1.99 (1.14 – 3.48)	200.8 ± 24.6	90.2 ± 16.3	45.3
GOO	0.91 (0.67 – 1.24)	535.4 ± 64.4	166.1 ± 31.9	182.5
GUN	0.97 (0.95 – 1.00)	1087.8 ± 160.9	317.2 ± 41.7	327.0

HAEMATOLOGY AND BIOCHEMISTRY

EFFECT OF SEX

Due to an inability to sex juvenile Eastern Grey Kangaroos from a distance by spotlight, 60% of the kangaroos sampled from GOO and GUN were sub-adult males. One kangaroo sampled at LMF was also male. No significant sex-related differences were detected for haemoglobin, haematocrit, red cell count, urea, albumin, triglyceride, or *Globocephaloides trifidospicularis* count (all $p > 0.4$). Females had significantly higher total protein (females, 52.63 g/L; males, 46.77 g/L; $p = 0.039$) and globulin (females, 19.59 g/L; males, 12.38 g/L; $p = 0.030$) than males. Females also had higher creatinine compared to males (females, 118.14 U/L; males, 97.31 U/L) although this trend was non-significant ($p = 0.06$). From these results it is possible that the population average for creatinine,

globulin and total protein at GOO and GUN may appear slightly lower due to the inclusion of males in the sampling regime.

EFFECT OF AGE

There was no significant difference ($p > 0.1$) in the average age of kangaroos across the four study sites although kangaroos at GUN (mean age 15.5 mo) were younger than those at GOO (17.2 mo), TNR (19.9 mo) and LMF (18.2 mo). No effect of age was observed for haemoglobin or haematocrit, however red cell count decreased slightly with age ($p = 0.047$). Creatinine ($p < 0.001$), total protein ($p = 0.004$) and globulin ($p < 0.001$) increased significantly with increasing age across all sites but urea, albumin and triglycerides ($p > 0.3$) did not.

EFFECT OF SITE

Significant differences were observed between sites for haemoglobin, haematocrit, red cell count, creatinine, albumin, globulin and triglyceride (all $p < 0.01$) (Figure 1). Haemoglobin, haematocrit and red cell count were all higher in 'low' kangaroo density sites (GOO, GUN) compared to 'high' kangaroo density sites (TNR, LMF) ($p < 0.01$). A similar pattern was observed for albumin although the differences between GOO and the high density sites were marginal (GOO vs LMF: $p = 0.056$; GOO vs TNR: $p = 0.048$; GUN vs TNR and LMF both $p < 0.015$). Globulin levels were significantly higher at TNR compared to other sites ($p < 0.003$). Creatinine was lower at GUN ($p < 0.05$) and higher at TNR compared to all other sites ($p < 0.001$) but levels were comparable between GOO and LMF ($p > 0.4$).

RELATIONSHIP WITH BODY WEIGHT AND KIDNEY AND BONE MARROW FAT

There was no relationship between haemoglobin, haematocrit, red cell count, urea, creatinine, total protein, albumin, globulin, triglycerides (all $p > 0.1$) and body weight. Trends indicating positive relationships were observed between kidney fat measurements and haemoglobin ($p = 0.07$) and haematocrit ($p = 0.09$), and negative relationships between kidney fat measurements and urea ($p = 0.06$) and triglycerides ($p = 0.07$) however these did not reach statistical significance.

There were significant positive relationships between bone marrow fat and haemoglobin ($p < 0.001$), haematocrit ($p < 0.001$), red cell count ($p = 0.004$), total protein ($p = 0.022$) and albumin ($p < 0.001$), and significant negative relationships between bone marrow fat and urea ($p = 0.011$) and triglycerides ($p = 0.012$) (Figure 2).

RELATIONSHIP WITH *GLOBOCEPHALOIDES TRIFIDOSPICULARIS* INFECTION INTENSITY

Haemoglobin ($p < 0.001$), haematocrit ($p < 0.001$), red cell count ($p < 0.001$) and albumin ($p = 0.012$) were negatively correlated with *Globocephaloides trifidospicularis* counts across all sites (Figure 3).

Figure 1. Relationships between site and haemoglobin (HB), haematocrit (Hct), red cell count (RCC), creatinine, albumin, globulin, triglyceride and *Globocephaloides trifidospicularis* count.

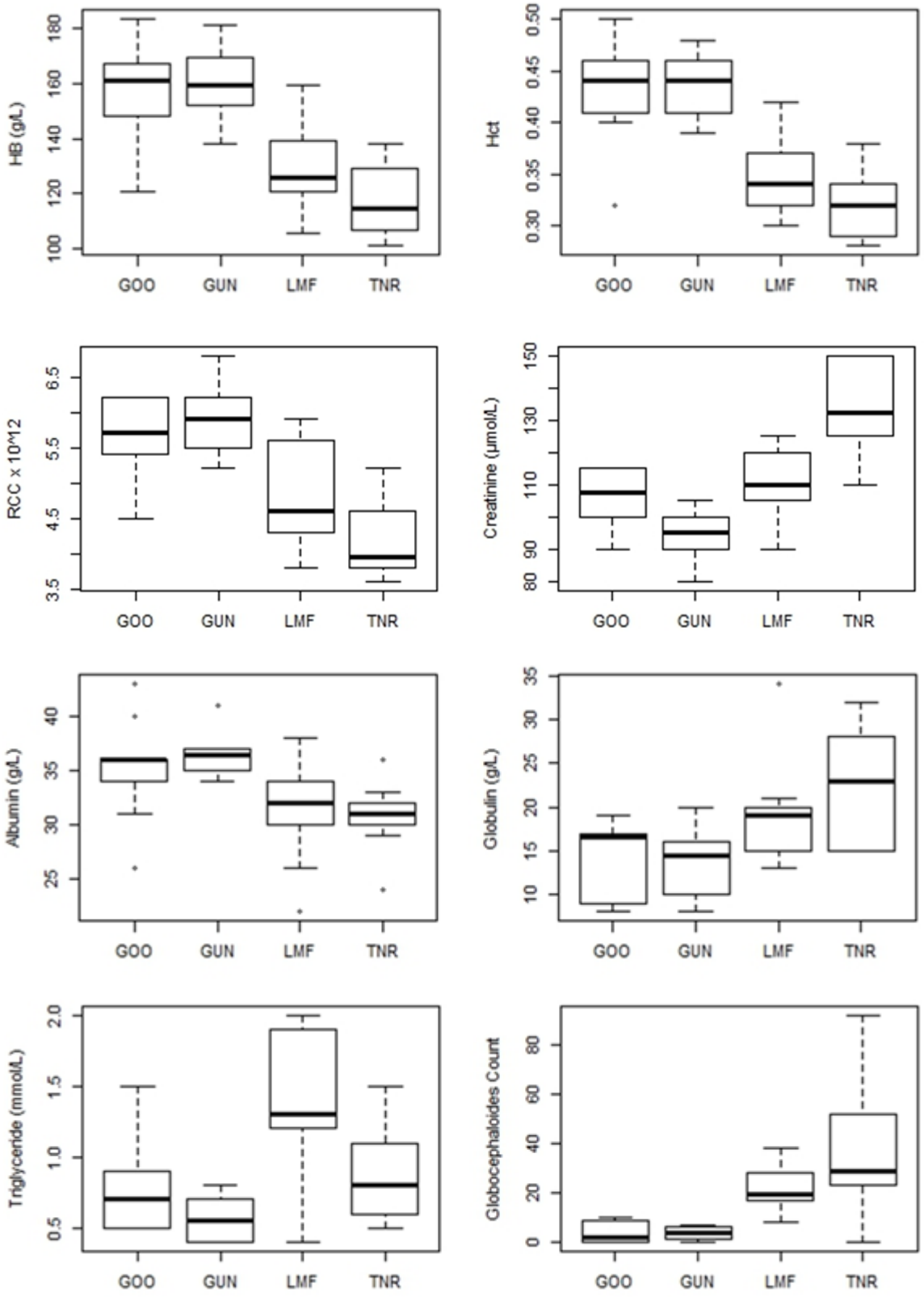


Figure 2. Relationships between bone marrow fat and haemoglobin, haematocrit, red cell count, blood urea nitrogen (urea), total protein, albumin, and triglycerides. GOO, ●; GUN, ●; LMF, ●; TNR, ●.

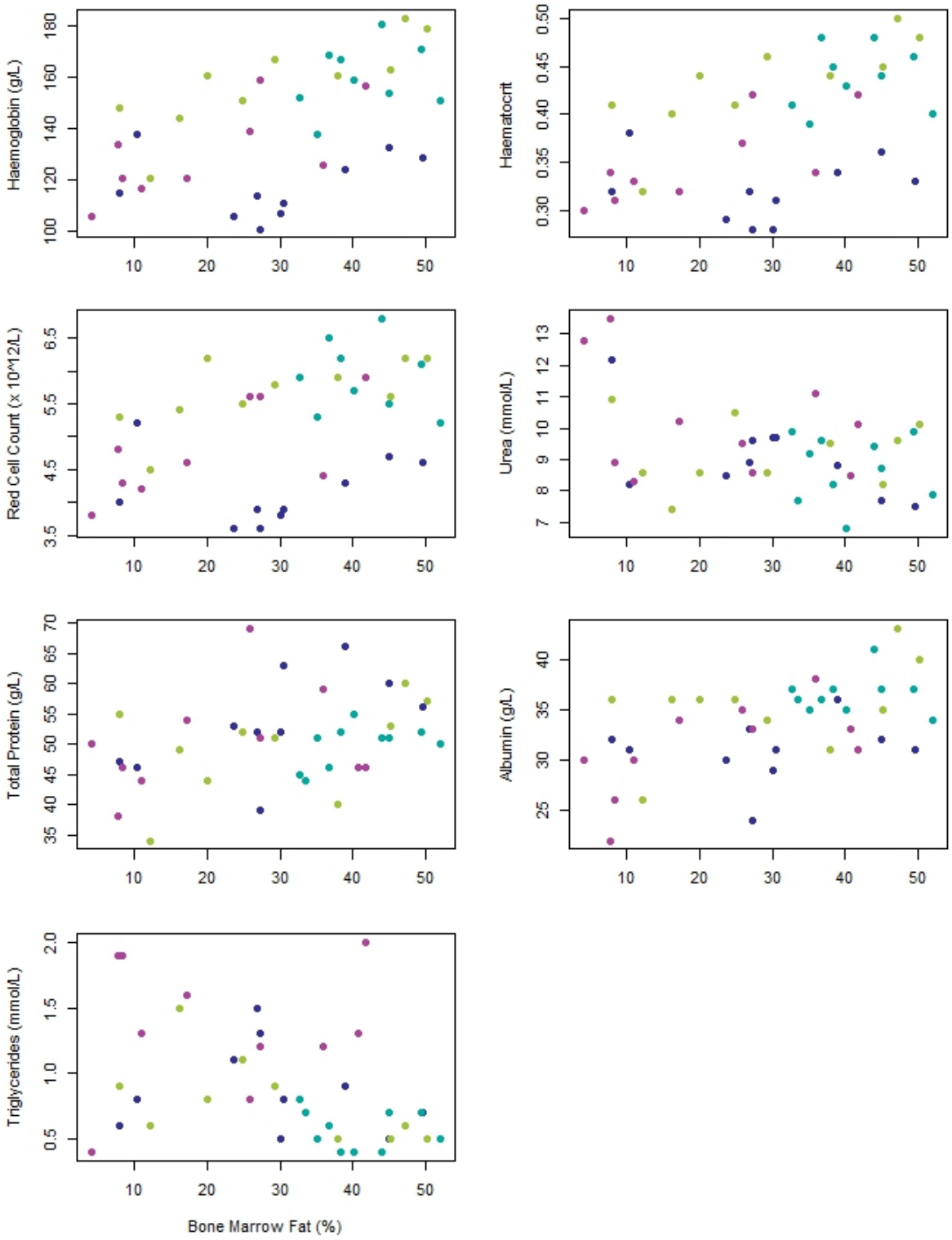
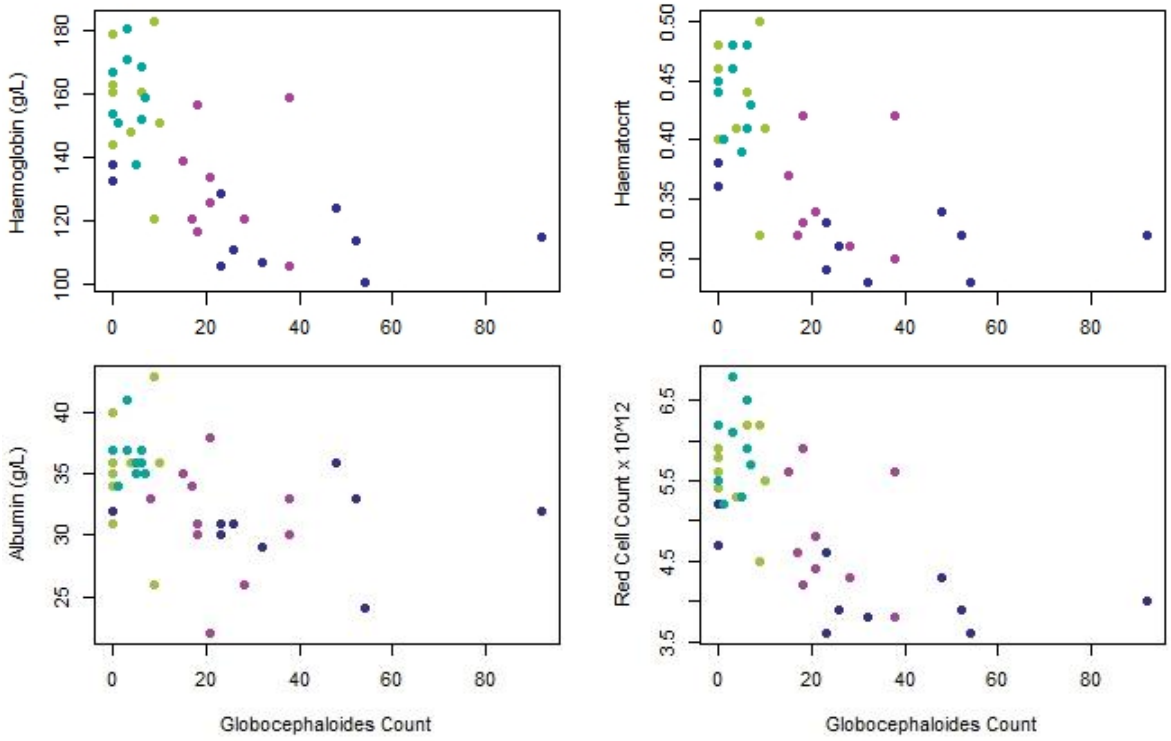


Figure 3. Relationships between *Globocephaloides trifidospicularis* count and haemoglobin, haematocrit, red cell count and albumin across the four study sites. GOO, ●; GUN, ●; LMF, ●; TNR, ●.



KANGAROO AGE, BODY WEIGHT, KIDNEY AND BONE MARROW FAT

No effect of sex was detected on the percentages of kidney or bone marrow fat measured in individuals, nor in their tail circumference or body weight ($p > 0.6$). Age was positively correlated with both the percentages of bone marrow ($p = 0.035$) and kidney fat ($p = 0.017$) in this study.

There was no difference in body weight or tail circumference of individuals across sites ($p > 0.3$). The effect of site on fat levels was marginal for kidneys ($p = 0.061$) and significant for bone marrow ($p = 0.005$) due to a tendency for a higher percentage of fat in animals from GUN compared to those from LMF (kidney, $p = 0.058$; bone marrow, $p = 0.015$) (Figure 4).

Increased percentages of both kidney and bone marrow fat were also associated with decreased numbers *Globocephaloides trifidospicularis*, although this relationship was not significant for kidney fat (kidney, p = 0.054; bone marrow, p = 0.019) (Figure 5).

Figure 4. Relationships between study site and body weight, tail circumference, % kidney fat and % bone marrow fat.

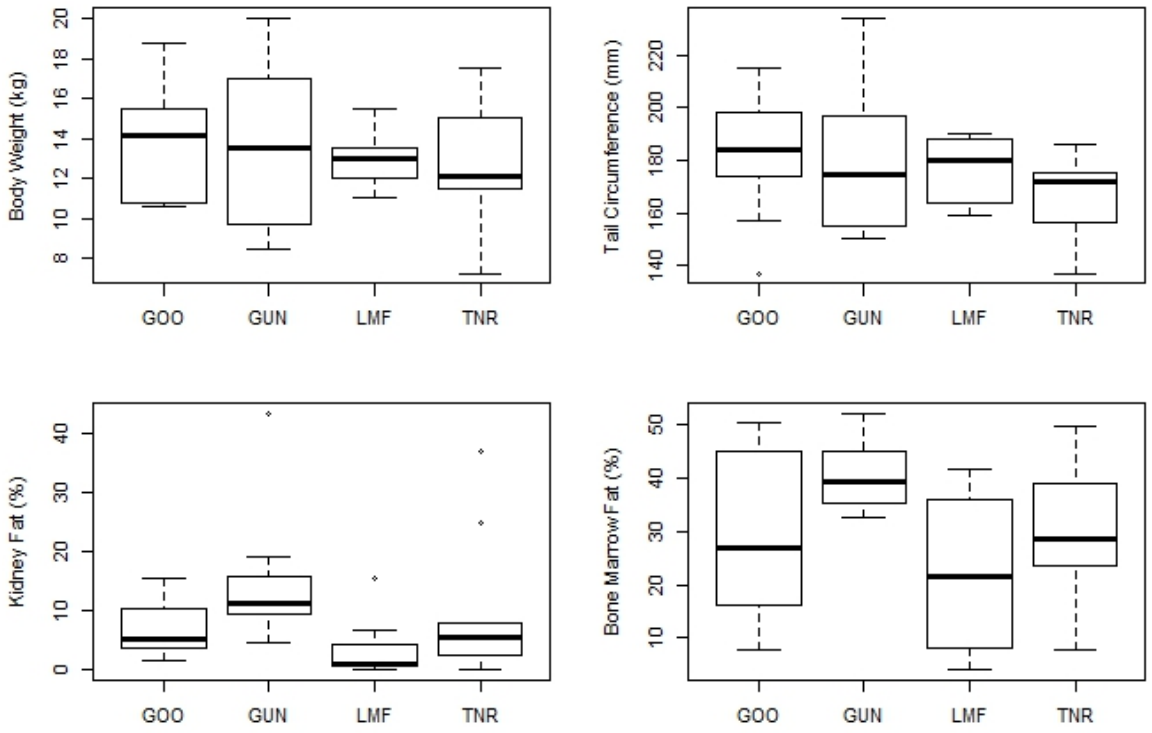
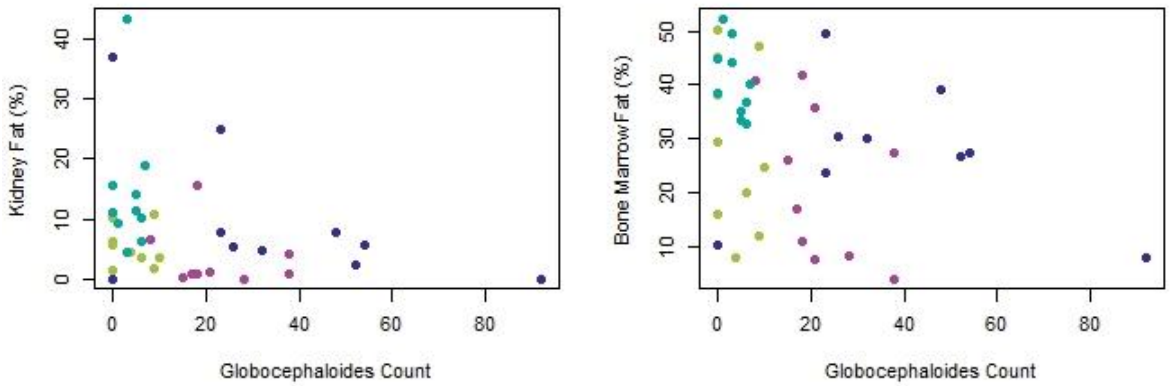


Figure 5. Relationships between *Globocephaloides trifidospicularis* count and % of kidney or bone marrow fat across the four study sites. GOO, ●; GUN, ●; LMF, ●; TNR, ●.



DISCUSSION

Variable kangaroo population densities at the sites in this study can be explained by management practices in which kangaroos are culled annually to achieve specific population densities for biodiversity conservation at some sites (GOO, GUN) and not others (LMF, TNR). Variations in herbage mass between sites are most likely influenced by the grazing pressure exerted by variable kangaroo population densities although somewhat unexpectedly there was not a significant difference in herbage mass between GOO, a low density site, and TNR, a high density site; possibly due to the presence of some larger unpalatable native tussocks at TNR. Temperature and rainfall are also known to affect herbage mass conditions through their influence on grass growth rates, however pasture growth is generally negligible in the ACT during the period covered by this project (July to September). Weather stations installed to estimate differences between sites had a high failure rate and so drawing useful inferences from these data was problematic.

This study demonstrated significant differences in a range of haematological and biochemical variables, percentage of bone marrow fat and *Globocephaloides trifidospicularis* infection intensity across four separate sites in the ACT. The differences were most pronounced between sites where high density kangaroo populations occurred and those where low density populations occurred. Broadly, differences in selected haematological and biochemical variables and the percentage of bone marrow fat between high and low density populations were suggestive of a higher plane of nutrition for kangaroos at low population density sites.

HAEMATOLOGY AND BIOCHEMISTRY

The differences in haemoglobin, haematocrit, red cell count and albumin observed between all sites can be explained, at least in part, by variations in herbage mass and per capita green herbage mass (the amount of green grass available per kangaroo) which likely influenced the nutritional status of kangaroos and variable *Globocephaloides trifidospicularis* infection intensity (see below). Differences in creatinine values between sites can be potentially explained by the differences in the mean ages of kangaroos at the various sites. While the age differences were not significant creatine values were highest at TNR where animals were oldest and lowest at GUN where animals were youngest. The increased globulin values at TNR may also be accounted for by the greater mean age of animals at TNR compared with other sites as this parameter increased significantly with increasing age.

Neither body weight nor kidney fat were significantly correlated with haematological or biochemical variables potentially indicative of nutritional status (haemoglobin, haematocrit, red cell count, urea, creatinine, total protein, albumin, globulin, triglycerides) although fat levels are expected to be low in all animals of this age group, regardless of nutritional state. However bone marrow fat was positively correlated with haemoglobin, haematocrit, red cell count, total protein and albumin suggesting that these variables may be useful measures of nutritional status in sub-adult Eastern Grey Kangaroos. This finding is broadly in line with other macropod species although species specific differences are evident (Stirrat 2000; Robert and Schwanz 2013; Portas et al., 2016). Kidney fat has been used as a measure of kangaroo body condition/nutritional status but Shepherd (1987) recommended its use be limited to adult kangaroos due to variable peri-renal fat deposition in response to changing nutritional conditions in younger animals.

GLOBOCEPHALOIDES TRIFIDOSPICULARIS

We used *Globocephaloides trifidospicularis* as a proxy for nematode infection intensity and prevalence rather than total worm counts. Species level identification of macropod nematodes requires considerable expertise and performing total worm counts is extremely resource intensive. Additionally, gastrointestinal parasite communities (excluding *Globocephaloides trifidospicularis*) have been shown to have little effect on haematological and biochemical variables in juvenile Eastern Grey Kangaroos other than reduced albumin levels (Cripps et al. 2014). Prevalence of infection with *Globocephaloides trifidospicularis* was lower than but comparable to previously reported in free-ranging Eastern Grey Kangaroos (Arundel et al. 1990). In this study higher *Globocephaloides trifidospicularis* counts were associated with lower haematocrit, haemoglobin, red cell count and albumin values. These findings are similar to those of Arundel *et al*. (1990) although in that study infection intensity was much higher. Additionally, increasing *Globocephaloides trifidospicularis* infection intensity was associated with lower kidney and bone marrow fat in the current study. Given the low infection intensity in kangaroos in the ACT and the fact that haematocrit, haemoglobin, red cell count, albumin (Stirrat 2000) and kidney and marrow fat can all be influenced by nutrition it is difficult to draw conclusions on the relative contribution of *Globocephaloides trifidospicularis* to variations in these parameters.

The differences in infection intensity at the different study sites can be explained by differences in kangaroo population densities and herbage mass between sites. Higher density sites will have higher pasture contamination with eggs of *Globocephaloides trifidospicularis* due to greater faecal contamination. Additionally, lower herbage mass at high population density sites will increase the risk of ingestion during grazing by kangaroos.

CONCLUSIONS

The scope of this study was limited to evaluating the health of sub-adult Eastern Grey Kangaroos during a defined interval in a single year. As such we were unable to determine potential factors that might account for periodic increased mortality in some populations of Eastern Grey Kangaroos in the ACT. However lower haemoglobin, haematocrit, red cell count and albumin at high density population sites compared with low density population sites demonstrates that the health of sub-adult Eastern Grey Kangaroos is influenced by population density. While these effects maybe subclinical under normal circumstances, affected animals are likely to be less resilient and more susceptible to environmental perturbations which further reduce pasture availability or increase energy requirements. In summary:

- There were significant differences between high and low kangaroo population density sites in the ACT for haemoglobin, haematocrit, red cell count, creatinine, albumin, globulin and triglyceride in sub-adult Eastern Grey Kangaroos.
- Differences between sites in the ACT for haemoglobin, haematocrit, red cell count and albumin in sub-adult Eastern Grey Kangaroos were most likely the result of variable pasture availability between sites, although infection intensity of *Globocephaloides trifidospicularis* may have played a part.

- Bone marrow fat was positively correlated with haemoglobin, haematocrit, red cell count, total protein and albumin suggesting that these variables may be useful measures of nutritional status in sub-adult Eastern Grey Kangaroos.
- Kidney fat does not appear to be a reliable indicator of nutritional status in sub-adult Eastern Grey Kangaroos in the ACT.
- Sub-adult Eastern Grey Kangaroos at low population density sites have physiological indicators of better nutritional status than those at high population density sites.

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