Reference intervals for parameters of health of eastern grey kangaroos Macropus giganteus and management implications across their geographic range

Authors: Brandimarti, Maquel E., Gray, Rachael, Coulson, Graeme, Cripps, Jemma K., Wilson, Michelle E., et al.

Source: Wildlife Biology, 2020(3)

Published By: Nordic Board for Wildlife Research

URL: https://doi.org/10.2981/wlb.00692

Wildlife Biology 2020: wlb.00692
doi: 10.2981/wlb.00692

Subject Editor: Aage K. Olsen Alstrup. Editor-in-Chief: Ilse Storch. Accepted 19 May 2020

Reference intervals for parameters of health of eastern grey kangaroos *Macropus giganteus* and management implications across their geographic range

Maquel E. Brandimarti, Rachael Gray, Graeme Coulson, Jemma K. Cripps, Michelle E. Wilson, Clare Death, Melissa Snape, Claire Wimpenny, Fabiola Rodrigues Oliveira Silva, Emily J. Miller, Elliot Scanes, Derek Spielman, Georgia Thomas and Catherine A. Herbert

M. E. Brandimarti (https://orcid.org/0000-0002-4055-6623) ✉ (maquel.brandimarti@sydney.edu.au), G. Thomas, C. A. Herbert (https://orcid.org/0000-0003-0174-0898), F. R. O. Silva and E. Scanes (https://orcid.org/0000-0001-7520-3804), School of Life and Environmental Science, The Univ. of Sydney, Camperdown, New South Wales 2006, Australia. – G. Coulson (https://orcid.org/0000-0001-9873-8203), J. K. Cripps (https://orcid.org/0000-0003-3049-5510) and M. E. Wilson, School of BioSciences, The Univ. of Melbourne, Melbourne, Victoria, Australia. JKC also at: Dept of Environment, Land, Water and Planning, Arthur Rylah Inst. for Environmental Research, Heidelberg, Victoria, Australia. – C. Death, Faculty of Veterinary and Agricultural Sciences, The Univ. of Melbourne, Werribee, Victoria, Australia. – M. Snape and C. Wimpenny, Conservation Research, Environment, Planning and Sustainable Development Directorate, ACT Government, Canberra, ACT, Australia. – E. J. Miller, The Univ. of Sydney, Camperdown, New South Wales, Australia. – R. Gray and D. Spielman, Sydney School of Veterinary Science, The Univ. of Sydney, Camperdown, New South Wales, Australia.

Reference intervals (RIs) describe baseline parameters of healthy animals, providing a powerful tool for wildlife managers to monitor health, identify disease and assess animal welfare. This paper reports haematological, glucose and serum protein RIs for one of Australia's most iconic and managed mammals, the eastern grey kangaroo *Macropus giganteus*. Blood samples (n = 514) were collected from 11 populations of eastern grey kangaroos, across much of their geographic range. A species-level RI was initially established based on samples collected from four sites (n = 245) and was further partitioned based on significant differences associated with sexual maturity and season. Unique population means were established from a further seven sites to investigate the importance of biotic (sex and sexual maturity) and abiotic (season, site, rainfall, temperature and laboratory) factors on kangaroo health parameters. Random forest analysis of health parameters revealed that abiotic factors (site, rainfall, temperature and season) were largely responsible for differences in haematological, glucose and serum protein values. Sex was found to have no influence, while sexual maturity and laboratory of analysis had moderate effects. Based on these findings, interpretation of individual and population haematological and serum protein values requires careful consideration of the timing of sample collection, environmental conditions and sexual maturity. When assessing kangaroo health, the relevant sexual maturity RI must be considered initially. For populations with similarities to those described (for example high density or captive populations) users should also consider site specific mean haematological and serum protein values. The RIs reported are valuable when establishing the health status of kangaroo populations. Furthermore, understanding the influence of biotic and abiotic factors will improve the utility of these RIs to assess health, disease status and improve welfare in eastern grey kangaroos.

Keywords: health, kangaroo, macropod, management, marsupial, reference intervals, welfare, wildlife

Haematological and serum protein parameters are routinely evaluated to assess health and detect disease in a wide range of species (Thrall et al. 2012). Individual blood values can be interpreted objectively and accurately by comparing with a reference interval (RI) developed from a 'healthy' cohort of the same species (Clark 2004, Friedrichs et al. 2012). RIs are increasingly used to monitor the health of wildlife, particularly in threatened species (Peck et al. 2015, Warren et al. 2015, Woolford et al. 2020) because sampling can be repeated with minimal impact on an animal's survival (Clark 2004). RIs are also helpful in assessing dietary shifts, nutritional stress and can predict survival when reintroducing or translocating animals (Mathews et al. 2006, Maceda-Veiga et al. 2015). Changes in serum protein concentrations

can indicate and differentiate between different parasite infections (Kaymaz et al. 1999) or can reflect energy and protein deficiencies (Robert and Schwanz 2013), assisting in diagnosis and management of animal health.

Kangaroos are a common marsupial found throughout Australia. Eastern grey kangaroos *Macropus giganteus* are one of the largest kangaroo species, with adult males weighing up to 85 kg (Coulson 2008). Eastern grey kangaroos occupy grassy woodlands throughout eastern and south-eastern Australia. Artificially high kangaroo densities now occur subsequent to forest clearing, improved pasture and removal of natural predators (Banks et al. 2000, Descovich et al. 2016). These high density kangaroo populations are subject to increased risk of density-dependent diseases, starvation and mass mortality (Portas and Snape 2018). Kangaroo body condition can be determined by palpating and visually assessing tail circumference/fat cover, evaluating kidney fat or bone marrow reserves, or by body condition scoring (Shepherd 1987, Moss and Croft 1999, Fletcher 2007). However, these options are destructive and/or subjective, and can have limited repeatability. Body condition indices using standardised residuals (King et al. 2011, Quesnel et al. 2017) or the 'scaled mass index' (Cripps et al. 2014, Gélin et al. 2015) can be derived from the ratio of body mass to skeletal length (Moss and Croft 1999). Condition indices are non-destructive, objective and repeatable, but like all current body condition assessments their comparative application across sites has not been tested. The management and harvesting of kangaroo populations can be controversial issues in Australia (Descovich et al. 2016). One of the rationales for kangaroo management is to minimise harm to the health and welfare of individual kangaroos in dense populations (Herbert 2004). Therefore, developing an objective tool to assess kangaroo health and welfare would be useful in kangaroo management.

There are no published haematological or serum protein RIs developed from free ranging eastern grey kangaroos. Although several authors (Clark 2004, Vogelnest and Portas 2008, Wilcox et al. 2011, Cripps et al. 2014, Green-Barber et al. 2018) have published haematological values for kangaroos, it may be inappropriate to apply them to other populations, as they could reflect local variation rather than provide baseline values for the species (Presidente 1978). In addition, some published haematological values have been developed under non-standard circumstances. For example; samples from captive populations, samples collected within a short timeframe or samples lacking individual and population demographics, such as sex, sexual maturity or season. Furthermore, blood values reported to date are based on sample sizes (maximum n = 53 from previous studies) well below those recommended for reliable RI development ($\geq$ 120) (Friedrichs et al. 2012). Additionally, the sources of biological and laboratory variabilities need to be examined to develop RIs as recommended by the American Society for Veterinary Clinical Pathology (ASVCP) (Friedrichs et al. 2012). Other recommendations for developing RIs include representative sampling across different demographic classes of the population, including only healthy individuals and standardised sample collection, handling and processing consistent for the species (Friedrichs et al. 2012). Given the often contentious nature of kangaroo management in Australia, it is important to ensure that RIs for the species are robust and that the potential effects of biotic and abiotic factors on RIs are well understood. This will enable wildlife managers to incorporate haematological parameters into evidence-based decisions on the health status of kangaroo individuals and populations.

This study provides comprehensive haematological RIs for the eastern grey kangaroo (hereafter kangaroo) and RIs for serum total protein, albumin, globulin and glucose concentrations. This study also consolidates independently collected mean haematological, glucose and serum protein values from 11 discrete populations of kangaroos spanning more than 1000 km of the species' geographical range (Fig. 1) determined over several years. The influence of biotic (sex and sexual maturity) and abiotic (season, site, rainfall, temperature and laboratory) factors on haematological, glucose and serum protein parameters are also examined using a novel statistical approach.

Material and methods

Data origin and site-specific details

Blood samples were collected from kangaroos at 11 sites across the species' geographic range (Table 1, Fig. 1). To meet the criteria for developing RIs, Nelson Bay Golf Course (NBGC), Darlington Park (DP), Heritage Park (HP) and Ainslie Majura Kangaroo Management Unit (AM) were selected as reference sites because only healthy animals were sampled, each site had a large and representative sample size (both males, females, adults and sub-adults) and all samples were analysed at the same laboratory as recommended by the ASVCP (Friedrichs et al. 2012). Blood samples (n = 245) were collected from these four reference sites between 2015 and 2019. However, not every parameter was analysed for each blood sample, so the sample size for some variables differ.

Separate from the RIs developed, site-specific mean haematological, glucose and serum protein values for the species are described by combining our reference sites with data collected independently by several researchers across Australia. Data from these additional sites were collected for disease investigations, management interventions and contraceptive efficacy trials. These sites are Anglesea Golf Course (AGC; Cripps et al. 2013, 2014, 2016, Wilson and Coulson 2016), Serendip sanctuary (SS; Borland et al. 2012, Wilson et al. 2013), Woodlands Historic Park (WHP; Coulson 2001, Coulson et al. 2008), Plenty Gorge Parklands (PGP; Wilson et al. 2013), Portland Aluminium (PA; Death et al. 2017), Cowan (CW; Miller et al. 2010) and Calga (CL) (Table 1, Fig. 1).

Sample collection

Kangaroos were immobilised or opportunistically sampled from culling operations. Immobilised kangaroos were sedated using Zoletil 100 (Virbac Pty. Ltd, Milperra, Australia) at a fixed dosage of 125 mg for females or sub-adult males and females, and 250 mg for adult males. Zoletil was

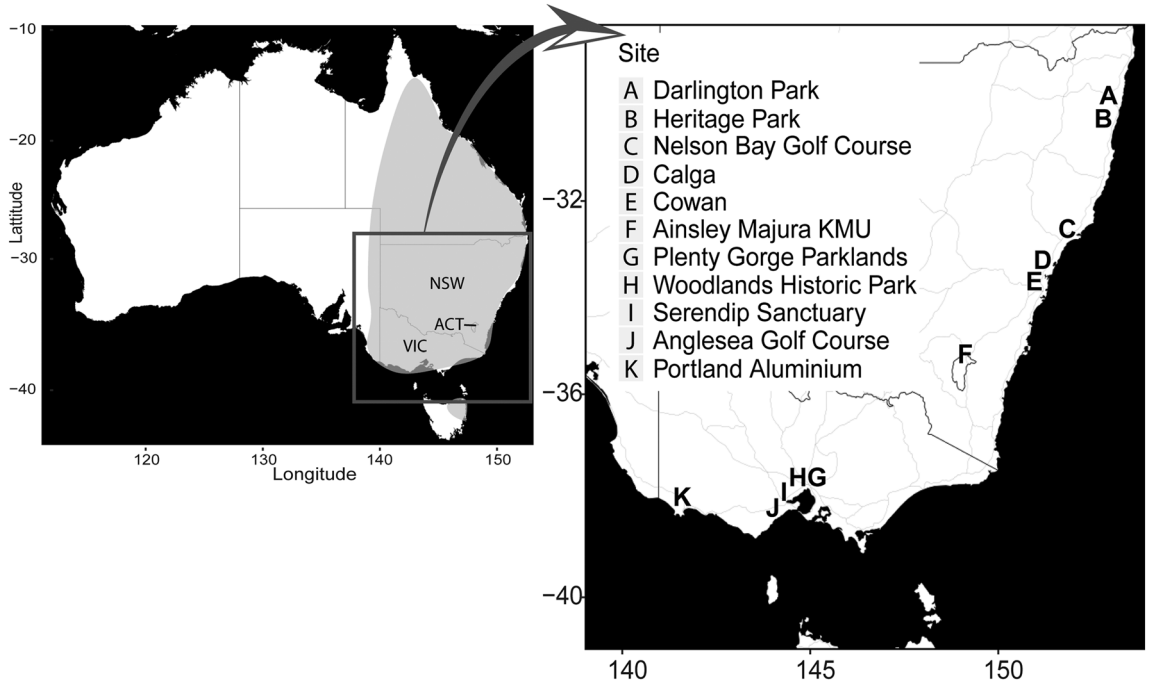

Figure 1. Geographic location of sampled eastern grey kangaroo *Macropus giganteus* populations in New South Wales (NSW), Nelson Bay Golf Course, (NBGC), Darlington Park (DP), Heritage Park (HP), Cowan (CW) and Calga (CL); Australian Capital Territory (ACT), Ainslie Majura Kangaroo Management Unit (KMU) (AM); Victoria (Vic), Anglesea Golf Course (AGC), Serendip Sanctuary (SS), Woodlands Historic Park (WHP), Portland Aluminium (PA). The grey shaded area represents the approximate distribution of eastern grey kangaroos. Populations were sampled from 2006 to 2019. The map of Australia is sourced from Stamen Design, under CC BY 3.0. Data by OpenStreetMap, under ODbL.

delivered via pole syringe or dart gun (X-Calibre, Pneudart Inc, Williamsport, PA, USA), as a 1 ml injection intramuscularly. Young-at-foot received a dose of approximately 65 mg as a 0.5 ml injection. Once immobilised kangaroos were transferred into a capture bag and transported to an onsite processing area (<1 km from all capture locations). During immobilisation, sex, pes and leg length (Poole et al. 1982), weight (Wedderburn Digital Hanging Scales, model WS603) and female reproductive status (pouch young, lactating, young-at-foot, non-reproductive) were recorded. Female maturity was determined by teat eversion (adult female) and reproductive status (presence of a pouch young). Male maturity was determined by leg length; > 52.3 cm were deemed sexually mature (Poole 1973, Poole et al. 1982). Each kangaroo was examined clinically for visible injuries and body condition. Up to 6 ml of whole blood was collected from the lateral caudal vein, approximately 30 min after immobilisation using a 20–22 gauge butterfly catheter attached to a 5 ml syringe.

Kangaroos from culling operations were dispatched by authorised shooters and transported to a central processing site on a trailer bed. Fresh carcasses were randomly selected and processed immediately. Whole blood was collected by cardiac venipuncture using an 18 gauge needle and attached 5 ml syringe. Most blood samples were collected within 5 min of death; the maximum time for blood collection post-death was 30 min.

For both immobilised and culled animals, blood was immediately transferred into a 5 ml BD Vacutainer SST II Advance tube and 1.3 ml EDTA tube. Whole blood was gently inverted and stored on an ice brick prior to processing. Whole blood was allowed to clot, then centrifuged for 10 min at 3000 rpm. Serum was stored at −20°C after separation. Two blood smears were freshly made using whole blood preserved in EDTA. Blood smears were air dried, fixed in methanol and stained with Diff Quik (Lab Aids Pty Ltd). Whole blood preserved in EDTA was mixed with Streck Cell Preservative (1:1 ratio, Streck, Omaha, NE, USA) and stored at 4°C prior to analysis.

Haematological and glucose analyses for the reference populations

Once collected, EDTA whole blood samples preserved in Streck were analysed using a Sysmex XN1000i automated haematology analyser (Roche diagnostics, Australia) at the Veterinary Pathology Diagnostic Services (VPDS), Sydney School of Veterinary Science, The University of Sydney, New South Wales (NSW) within seven days of blood collection. Quality control of the analyser is maintained internally every day, and externally cyclically, by relevant quality assurance programs under the Royal College of Pathologists Australasia (RCPAQAP). The following parameters were determined: haematocrit (HCT; $l l^{-1}$), haemoglobin (HGB; $g l^{-1}$), total red blood cell count (RBC; $\times 10^{12} l^{-1}$), total white blood cell count (WBC; $\times 10^{9} l^{-1}$) platelet count (PLT; $\times 10^{9} l^{-1}$) and nucleated red blood cell count (NRBC; $\times 10^{9} l^{-1}$). Values were doubled to correct for dilution with cell preservative. Mean cell volume (MCV; fL; (HCT/1000)/RBC), mean cell haemoglobin (MCH; pg; (HGB/RBC)) and mean corpuscular haemoglobin concentration

Table 1. Kangaroo site, management and clinical history information from 11 discrete populations of eastern grey kangaroos *Macropus giganteus* across their geographic range in Australia; Nelson Bay Golf Course, (NBGC), Darlington Park (DP), Heritage Park (HP), Ainslie Majura Kangaroo Management Unit (KMU) (AM), Anglesea Golf Course (AGC), Serendip Sanctuary (SS), Woodlands Historic Park (WHP), Portland Aluminium (PA), Cowan (CW) and Calga (CL).

Nelson Bay Golf Course (NBGC)		
Sampling details	Years sampled	2015–2019
	Sample size	≤ 83
	Laboratory	Veterinary Pathology Diagnostic Services (VPDS)
	Capture/sampling type	Immobilised using Zoletil
Site information	Description	Peri-urban free ranging population on a 20 ha coastal golf course
	Habitat type	Pastoral grasses and dry sclerophyll vegetation
	Latitude and longitude	32°72′8″S, 152°15′0″E
	Clinical history	Good condition. Occasional known incidence of disease (trauma, lumpy jaw, tape worm). Primary known cause of death: motor vehicle collision (MVC)
Kangaroo management	Population density	1.88 (2015) to 1.21 (2018) individuals ha^{-1}
	Population trend	Declining
	Management intervention	Fertility control (Deslorelin contraceptive implants) applied in 2013 to 39 females
Darlington Park (DP)		
Sampling details	Years sampled	2017–2019
	Sample size	≤ 59
	Laboratory	VPDS
	Capture/sampling type	Immobilised using Zoletil
Site information	Description	Semi-rural free ranging coastal population. Animals based in caravan park, golf course, private farmland and coastal bushland
	Habitat type	Wet and dry sclerophyll forest, ornamental grasses and grazing pasture
	Latitude and longitude	30°04′8″S, 153°19′1″E
	Clinical history	Good condition. Primary known cause of death is MVC
Kangaroo management	Population density	1.44 individuals ha^{-1}
	Population trend	Static
	Management intervention	Fertility control (Deslorelin contraceptive implants) applied in 2017 to 22 females
Heritage Park (HP)		
Sampling details	Years sampled	2017
	Sample size	≤ 64
	Laboratory	VPDS
	Capture/sampling type	Immobilised using Zoletil
Site information	Description	Semi-rural free ranging population. Animals based in a newly developed housing estate, historically agricultural land
	Habitat type	Grazing pasture/grasslands and wet and dry sclerophyll forests
	Latitude and longitude	30°18′3″S, 153°14′9″E
	Clinical history	Animals in good condition. Occasional reports of sub-adult kangaroo mortality. MVC is the primary known cause of death
Kangaroo management	Population density	1.23–1.52 individuals ha^{-1} (Henderson et al. 2018)
	Population trend	Increasing
	Management intervention	Fertility control (Deslorelin contraceptive implants) applied in 2017 to 45 females
Ainslie Majura Kangaroo Management Unit (KMU) (AM)		
Sampling details	Years sampled	2018
	Sample size	≤ 28
	Laboratory	VPDS
	Capture/sampling type	Cull
Site information	Description	Semi-rural free ranging population within Mount Ainslie Nature Reserve
	Habitat type	Natural temperate grassland and box-gum grassy woodland
	Latitude and longitude	35°27′4″S, 149°16′5″E
	Clinical history	Good condition based on the amount of renal fat collected from fresh carcasses
Kangaroo management	Population density	1.69 individuals ha^{-1} (before 2018 cull)
	Population trend	NA
	Management intervention	Culling conducted in 2018
Anglesea Golf Course (AGC)		
Sampling details	Years sampled	2008–2012
	Sample size	≤ 60
	Laboratory	IDEXX
	Capture/sampling type	Immobilised using Zoletil

(Continued)

Table 1. Continued.

Site information	Description	Peri-urban, free ranging population on a 73 ha golf course
	Habitat type	Pastoral grasses and dry sclerophyll woodland with a shrub understorey
	Latitude and longitude	38°40′6″S, 144°17′1″E
	Clinical history	Good condition. Occasional known incidence of disease (trauma, lumpy jaw). Primary cause of death is MVC
Kangaroo management	Population density	2.0 (winter) to 3.6 (summer) individuals ha^{-1}
	Population trend	Essentially stable
	Management intervention	Fertility control applied to adult females in 2008–2011: 24 treated with Deslorelin implants and 24 with Levonorgestrel implants. Anthelmintic treatments (Ivermectin, Moxidecin, Albendazole) administered to 40 adults in 2010–2011. Albendazole administered to 42 juveniles in 2012
Serendip sanctuary (SS)		
Sampling details	Years sampled	2007–2009
	Sample size	≤ 80
	Laboratory	IDEXX and Gribbles
	Capture/sampling type	Immobilised using Zoletil or cull
Site information	Description	Peri-urban, free ranging population in a 250 ha nature reserve with bird breeding and display areas
	Habitat type	Improved pasture, revegetated woodland, remnant dry sclerophyll woodland and ephemeral wetlands
	Latitude and longitude	38°00′3″S, 144°41′1″E
	Clinical history	Poor condition. Inadequate food, high incidence of MVC and extremely high prevalence of oral necrobacillosis (lumpy jaw)
Kangaroo management	Population density	1.1–2.6 individuals ha^{-1}
	Population trend	Declining
	Management intervention	Extensive culls conducted in 2007 and 2009. Fertility control (Deslorelin implants) applied to 18 adult females in 2007–2008
Woodlands Historic Park (WHP)		
Sampling details	Years sampled	2008
	Sample size	≤ 19
	Laboratory	IDEXX
	Capture/sampling type	Cull
Site information	Description	Peri-urban population in a 300 ha predator-proof enclosure
	Habitat type	Lowland native grassland and dry sclerophyll woodland with grassy or shrubby understorey
	Latitude and longitude	37°64′3″S, 144°53′1″E
	Clinical history	Generally good condition.
Kangaroo management	Population density	1.6 individuals ha^{-1}
	Population trend	Fluctuating
	Management intervention	Regular culls conducted. Fertility control (Levonorgestrel implants) applied to 25 adult females in 1999–2000
Plenty Gorge Parklands (PGP)		
Sampling details	Years sampled	2007
	Sample size	≤ 8
	Laboratory	IDEXX
	Capture/sampling type	Immobilised using Zoletil
Site information	Description	Peri-urban, free ranging population in a 1355 ha reserve
	Habitat type	Retired pasture, wetlands and dry sclerophyll woodland
	Latitude and longitude	37°62′9″S, 145°11′3″E
	Clinical history	Generally good condition
Kangaroo management	Population density	0.6 individuals ha^{-1}
	Population trend	Probably increasing
	Management intervention	Fertility control (Deslorelin implants) applied to 11 adult females in 2007–2008
Portland Aluminium (PA)		
Sampling details	Years sampled	2010–2013
	Sample size	≤ 74
	Laboratory	Gribbles
	Capture/sampling type	Immobilised using Zoletil or cull
Site information	Description	Peri-urban, free ranging population in a 450 ha buffer zone around an aluminium smelter
	Habitat type	Improved pasture (grazed by cattle), hardwood plantation, wetlands, coastal heathland and shrubland
	Latitude and longitude	38°38′3″S, 141°62′3″E
	Clinical history	Chronic fluoride exposure resulting in varying degrees of dental and skeletal fluorosis in most individuals; skeletal and dental lesions more severe in older cases, however generally good animal welfare and body condition, with minimal changes to other body systems noted on necropsy

(Continued)

Table 1. Continued.

Kangaroo management	Population density	~0.3 individuals ha^{-1}
	Population trend	Fluctuating
	Management intervention	Regular culls conducted. Fertility control (Levonorgestrel implants) applied to 18 adult females in 1999
Cowan (CW)		
Sampling details	Years sampled	2006–2007
	Sample size	≤ 22
	Laboratory	IDEXX
	Capture/sampling type	Immobilised using Zoletil
Site information	Description	Captive population at The University of New South Wales (UNSW) Research Facility. Colony was enclosed within 9 ha of natural bushland
	Habitat type	Dry sclerophyll forest and improved pasture
	Latitude and longitude	33°59′4″S, 151°15′6″E
	Clinical history	Good condition
Kangaroo management	Population density	NA
	Population trend	NA
	Management intervention	Supplementary feeding, bi-monthly intestinal parasite treatment (Equiban)
Calga (CL)		
Sampling details	Years sampled	2007
	Sample size	< 5
	Laboratory	IDEXX
	Capture/sampling type	Immobilised using Zoletil
Site information	Description	68 ha predator proof sanctuary of natural bushland
	Habitat type	Dry sclerophyll forest
	Latitude and longitude	33°42′4″S, 151°22′3″E
	Clinical history	Good condition
Kangaroo management	Population density	NA
	Population trend	NA
	Management intervention	Supplementary feeding

(MCHC; g l^{-1}; (HGB/HCT)) were calculated manually. Haemolysed samples collected from shot individuals were excluded from analysis.

One hundred WBCs were differentiated on stained blood smears to determine the percentage of neutrophils, lymphocytes, eosinophils, monocytes and basophils. Absolute WBC counts were determined by multiplying the percentage of each WBC type determined on the blood smear (%) by the total WBC count determined by the automated analyser (× 10^{9}l^{-1}). Blood glucose concentration was determined immediately after collection in the field. One to two drops of fresh blood was applied to a glucose strip (Freestyle optimum glucose strips, Abbott, Alameda, California) and blood glucose determined using a hand-held glucose monitoring device (Freestyle Optium Neo Blood Glucose Monitoring System, Abbott, Alameda, California).

Serum protein analysis for the reference population

Frozen serum was thawed at room temperature prior to analysis. Albumin (gl^{-1}), total protein (TP; gl^{-1}) and globulin (gl^{-1}) concentrations were determined using the Konelab Prime 30i analyser (Thermo scientific, Australia) at VPDS, within seven days of blood collection. Relevant external quality control measures were undertaken regularly (Randox, County Antrim, UK).

Statistical analyses

Species-level reference intervals of health parameters

Data analysis followed the methods outlined by the 2012 ASVCP guidelines for the development of RIs (Friedrichs et al. 2012). RIs were developed using R ver. 3.5.3 statistical software (<www.r-project.org>) in the 'referenceIntervals' ver. 1.1.1 R package (Finnegan 2015). Outliers were identified and removed using Horn's algorithm (using Tukey's interquartile fences on a Box–Cox transformation) or Cook's distance (for datasets containing zeros). Identified values using Cook's distance were confirmed visually as true outliers by evaluating a histogram of the data.

Based on the recommendations by the ASVCP (Friedrichs et al. 2012), non-parametric methods with 95% confidence intervals (CI) were used where n > 120 reference samples were available, or if alternative methods could not be applied. With > 40 and < 120 reference samples, robust methods with 90% CIs with bootstrapping (Friedrichs et al. 2012) were used on symmetrical data. For asymmetric data normality was assessed. If data were normally distributed, parametric analysis was performed. If data were not normally distributed, non-parametric methods were used and the mean (± standard deviation; SD) and median presented. When > 20 and < 40 reference samples were available, normally distributed data were analysed using parametric methods, and non-normal data were analysed using robust methods; minimum and maximum values presented. When < 20 reference samples were available, only the mean value (± SD) is presented. Basophil and NRBC counts contained too many zero values to calculate a RI; instead a range of observed values is presented.

Species-level RIs were created by combining data from both sexes (male and female), maturity (sub-adult, adult), sites (DP, HP, NBGC and AM) and seasons (summer, autumn, winter and spring). To minimise overrepresentation of a sub-group into the RI, each sex/maturity/site and season

combination was sampled equally (where possible). Statistical criteria to aid partitioning was employed for each factor (sex, season and sexual maturity). Normally distributed data (albumin, total protein and globulin) were partitioned using distance between reference limits of the sub-group distributions (Lahti et al. 2002). Non-normal data were partitioned based on the proportion of observations in the distributions of the sub-groups that fell outside of the combined RIs that were $\geq$ 4.1% or $\leq$ 0.9% (Lahti et al. 2004). Maturity RIs (sub-adult and adult) included reference samples from both sexes, sites and seasons. Seasonal RIs were created only for summer, autumn and winter due to the low sample size ($n < 20$) for animals sampled in spring.

Site-specific mean health parameters

Mean values ($\pm$ SD) were determined for each site using all data (including sex, maturity, season and sampling years).

Influence of biotic and abiotic factors on reference intervals and site-specific means

Significance and effect size of biotic and abiotic factors

Effect size (ES) was used to examine the size of the difference between two groups (Nakagawa and Cuthill 2007). ES benchmark values of 0.2, 0.5 and 0.8 were selected to indicate small, medium and large effect size respectively (Cohen 1988). ES were established using the 'effsize' and 'MBESS' R packages (R ver. 3.5.3). For biotic factors with two sets of observations (male versus female and sub-adult versus adult), ES was calculated using Cohen's *d* statistics (Durlak 2009), as the data contained both continuous and categorical data (Nakagawa and Cuthill 2007). A *d* value of 0.5 can be interpreted as the group means differing by 0.5 SDs. An ES of zero indicates there is no effect, whilst either a low or upper CI of zero (or near zero) indicates the sub-group mean differences are quite small. The ES for season was calculated using eta squared (η^2) which can be employed when there are more than two sets of observations (Lakens 2013, Maher et al. 2013). η^2 benchmark values of 0.01, 0.06 and 0.14 indicate small, medium and large ES respectively (Maher et al. 2013). A η^2 of 0.14 for season means that 14% of the total variance can be accounted for by season. CIs were calculated for both *d* and η^2 ES point estimates. Negative ES indicate a decrease in the parameter of interest, for example lower RBC count in female kangaroos, while positive ES indicate an increase. To facilitate comparison of ES among all variables, ES estimates (*d* or η^2) and 95% CIs of *d* and η^2 are depicted in a forest plot for each selected factor.

If the ES was categorised as medium to large, factorial significance was also calculated. To establish factorial significance, a Shapiro–Wilk normality test was initially performed for each haematological, glucose and serum protein parameter, within each factor. Where parameters were normally distributed ($p > 0.05$), the parameters were assessed using one-way analysis of variance (ANOVA). For parameters that were not normally distributed ($p < 0.05$), either a square, log or cube root transformation was performed before analysis using ANOVA. If a normal distribution of the data could not be achieved (on either native or transformed data) the non-parametric Kruskal–Wallis (KW) test was applied. The significance between sites was established using the same methodology.

Random forest model of factors influencing parameters of health

To explore the effects of biotic and abiotic factors on the haematological, glucose and serum protein values of kangaroos, random forest (RF) models were used to analyse parameters from all 11 sites. RF models have high accuracy with complex datasets, can incorporate multiple predictor variables, automatically handle missing data and are easy to apply and interpret (Cutler et al. 2007). RF models are also robust to collinearity, which precluded the use of linear models on this dataset. 18 models, one for each parameter, were created using the 'randomForest' R package. Each factor (site, season, sex, sexual maturity, laboratory, rainfall and temperature) was selected to train the model based on their known influence on haematological and serum protein parameters (Pacioni et al. 2013, Fancourt and Nicol 2019). Average monthly rainfall and temperature data were obtained from the nearest weather station (Bureau of Meteorology 2020) for the month preceding collection for each site. Samples were analysed by three commercial laboratories (VPDS, IDEXX Laboratories (IDEXX), NSW and Victoria (Vic) and Gribbles Veterinary Pathology (Gribbles), Vic), so laboratory was used as a factor in the RF model. RF models were grown using 1000 trees, with each tree using a bootstrap sample of 66% of the data. The number of variables tested at each split (mtry) was set at seven. Percentage of variation explained (PVE), and root mean square error (RMSE) were calculated for each model using 'out of the bag' predictions. A randomisation technique was used to test whether the model was significantly different to that of a random model (Murphy et al. 2010). Biotic and abiotic factors for each model were randomised and re-run 1000 times. The PVE from both the real (non-random data) model and the random model were compared and a p-value generated (Evans and Cushman 2009, Murphy et al. 2010) to evaluate model significance. Finally, the percent increase in mean square error (%IncMSE) was used to determine the importance of each selected biotic and abiotic factor on each haematological, glucose and serum protein parameter (Breiman 2001, Evans and Cushman 2009). The %IncMSE provides a measure of how much the predictive ability of the model is reduced when the effect of a certain parameter is excluded. To demonstrate the collective importance of each factor, the number of times a factor occurred in the top three %IncMSE for each parameter was recorded and summed to create a rank score (ascending in importance). To examine the effect of temperature and rainfall on haematological, glucose and serum protein values, partial dependence plots (PDPs) were generated for all parameters with a PVE greater than 50. Predicted effects from models lower than a PVE of 50 were not used in this analysis due to their potential lack of reliability (Evans et al. 2011).

Results

Species-level reference intervals of health parameters

Species-level haematological, glucose and serum protein RIs for the reference population are presented in Table 2.

Most parameters for each factor satisfied statistical criteria recommended for partitioning datasets into selected factors (Lahti et al. 2002, 2004). Maturity and season specific RIs are presented in Table 3, 4 respectively and are described in more detail.

Site-specific mean health parameters

Site-specific mean haematological, glucose and serum protein parameters are presented as descriptive box plots in Fig. 2 (and in Supplementary material Appendix 1 Table A1). Values were included from a maximum of n = 269 individuals. Differences among site means were dependent on the parameter examined.

Influence of biotic and abiotic factors on reference intervals and site-specific means

Significance and effect size of biotic and abiotic factors

Several haematological and serum protein parameters varied with the effects of sex, season and sexual maturity (Table 5). The ES of sex and maturity on all variables combined ranged from −0.76 to 0.42 (Table 5, Fig. 3a–b). Seasonal ES on all variables ranged from 0 to 0.34 (Table 5, Fig. 4). Mean ES were both positive and negative for sex and maturity, but always positive for season.

Sex

The ES of sex on variables was negligible for most parameters analysed, while a small effect of sex was seen for RBC count, monocyte count, basophil count, MCV, MCH and NRBC count. The ES of sex on glucose concentration (Table 5, Fig. 3b) was medium and shown to be significantly higher in males compared to females (ANOVA: $F_{1,127} = 20.91$, $p < 0.001$, Table 5), with means of 4.06 ± 0.96 mmol l^{-1} and 3.31 ± 1.3 mmol l^{-1} respectively. Due to the overall small effect of sex on haematological and protein parameters, RIs were not partitioned by sex.

Maturity

Adults had significantly lower glucose concentrations and lymphocyte counts than sub-adults (ANOVA: $F_{1,127} = 10.83$, $p < 0.01$ and $F_{1,197} = 12.52$, $p < 0.001$ respectively, Table 5), with a medium ES. Adults had significantly higher MCV (ANOVA: $F_{1,228} = 32.73$, $p < 0.001$), MCH (ANOVA: $F_{1,228} = 17$, $p < 0.001$), MCHC (ANOVA: $F_{1,228} = 4.72$, $p = 0.03$), TP (ANOVA: $F_{1,53} = 12.06$, $p < 0.01$) and globulin concentrations (ANOVA: $F_{1,51} = 18.98$, $p < 0.01$) than sub-adults, with a medium ES (Table 5). For all remaining parameters, there was a small ES of maturity, with no significant differences. Based on these results, RIs were partitioned by maturity and are presented in Table 3.

Season

Several variables were affected by season (Table 4). This effect was large and significantly different for RBC count (ANOVA: $F_{3,228} = 4.7$, $p < 0.001$), HCT (ANOVA: $F_{3,228} = 24.15$, $p < 0.001$), MCV (ANOVA: $F_{3,228} = 11.06$, $p < 0.001$), MCH (ANOVA: $F_{3,228} = 13.76$, $p < 0.001$), MCHC (ANOVA: $F_{3,228} = 25.26$, $p < 0.001$) and albumin concentration (ANOVA: $F_{3,94} = 19.01$, $p < 0.001$) (Table 5, Fig. 4). Glucose, monocyte count, HGB, PLT and NRBC count were also dependent on season, with a medium ES (Table 5, Fig. 4). Glucose (ANOVA: $F_{3,127} = 2.82$, $p = 0.04$), monocyte count (ANOVA: $F_{3,197} = 4.77$, $p < 0.01$), HGB (ANOVA: $F_{3,228} = 7.44$, $p < 0.001$) and PLT (ANOVA: $F_{3,228} = 4.26$, $p = 0.01$) were significantly different across seasons (Table 5). There was a small effect on neutrophil counts, lymphocyte counts and basophil counts, but there was no effect of season

Table 2. Non-parametric haematological, glucose and serum protein reference intervals for free ranging eastern grey kangaroos *Macropus giganteus* sampled from reference sites; Nelson Bay Golf Course, (NBGC), Darlington Park (DP), Heritage Park (HP) and Ainslie Majura Kangaroo Management Unit (KMU) (AM) from 2015 to 2019.

Parameter	Units	n	Lower reference interval (CI)	Upper reference interval (CI)	Mean (SD)	Median
RBC count	10^{12} l^{-1}	227	1.52 (1.34–1.7)	4.83 (4.58–5.49)	2.99 (0.87)	2.92
WBC count	10^{9} l^{-1}	223	2.79 (2.16–3.5)	13.1 (12.08–14.36)	7.1 (2.46)	6.9
Glucose	mmol l^{-1}	133	1.84 (1.7–2.2)	7.06 (5.6–8.9)	3.65 (1.21)	3.5
Neutrophil count	10^{9} l^{-1}	197	0.49 (0.45–0.68)	5.17 (4.87–5.7)	2.47 (1.16)	2.32
Lymphocyte count	10^{9} l^{-1}	194	1.56 (1.34–1.99)	8.3 (7.61–8.75)	3.85 (1.63)	3.47
Eosinophil count	10^{9} l^{-1}	196	0.04 (0)	1.39 (1.29–1.41)	0.56 (0.36)	0.51
Monocyte count	10^{9} l^{-1}	196	0 (0)	0.52 (0.45–0.65)	0.13 (0.15)	0.08
Basophil count	10^{9} l^{-1}	195	0 (0)[b]	0.09 (0.07–0.1)[b]	0.01 (0.02)	0
HGB	g l^{-1}	226	96.7 (92–102)	164 (158–170)	129.02 (16.7)	128
HCT	l l^{-1}	233	0.14 (0.09–0.16)	0.41(0.4–0.45)	0.27 (0.08)	0.27
MCV	fl	232	62.15 (59.18–63.4)	107.91 (105.51–108.99)	91.12 (11.46)	94.35
MCH	pg	232	22.42 (20.77–24)	74.02 (67.82–76.12)	44.9 (11.86)	43.22
MCHC	g l^{-1}	234	343.37 (327.59–349.36)	810.71 (718.31–1156.86)	497.72 (139.3)	468.77
PLT	10^{9} l^{-1}	218	66.95 (60–85)	286.1 (258–312)	155.94 (48.91)	151.5
NRBC count	10^{9} l^{-1}	71	0 (0)[b]	0.14 (0.14–0.16)[b]	0.03 (0.04)	0.02
Albumin	g l^{-1}	100	18.06 (16.11–19.89)	42.59[a] (40.92–44.23)	29.91 (6.14)	
TP	g l^{-1}	55	54.19 (51.79–56.46)	77.37[a] (75.35–79.8)	65.55 (5.68)	
Globulin	g l^{-1}	55	22.55 (20.54–24.57)	41.55[a] (40.1–43.19)	31.67 (4.67)	

RBC, red blood cell count; WBC, white blood cell count; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelets; NRBC, nucleated red blood cell count; TP, total protein; CI, confidence interval; SD, standard deviation.

[a] Calculated using robust methods.

[b] Range of observed values.

Table 3. Maturity specific haematological, glucose and serum protein reference intervals for adult and sub-adult free ranging eastern grey kangaroos *Macropus giganteus* sampled from reference sites; Nelson Bay Golf Course, (NBGC), Darlington Park (DP), Heritage Park (HP) and Ainslie Majura Kangaroo Management Unit (KMU) (AM) from 2015 to 2019.

Parameter	Units	Maturity	n	Lower reference interval (CI)	Upper reference interval (CI)	Mean (SD)	Minimum	Maximum	Median
RBC count	10^{12} l^{-1}	adult	178	1.49[b] (1.34–1.65)	4.51[b] (4.32–4.78)	2.87 (0.78)			2.82
		sub-adult	54	0.46[a] (0–1.14)	6.27[a] (5.61–6.97)	3.53 (1.41)			
WBC count	10^{9} l^{-1}	adult	173	1.78[b] (1.38–2.96)	11.88[b] (10.72–12.8)	6.93 (2.28)			6.96
		sub-adult	53	2.28[b] (1.4–2.56)	15.09[b] (14.83–16.62)	7.37 (3.26)			6.46
Glucose	mmol l^{-1}	adult	98	1.09[a] (0.71–1.52)	5.47[a] (5.03–5.91)	3.43 (1.09)			
		sub-adult	35	1.31[a] (0.37–2.24)	6.77[a] (5.89–7.78)	4.28 (1.32)	2.5	8.9	
Neutrophil count	10^{9} l^{-1}	adult	155	0.62[b] (0.45–5.24)	5.24[b] (4.99–5.74)	2.6 (1.15)			2.51
		sub-adult	42	0[a] (0–0.19)	4.1[a] (3.53–4.7)	2 (1.08)			
Lymphocyte count	10^{9} l^{-1}	adult	161	0.74[b] (0.63–1.51)	7.59[b] (6.95–8.31)	3.52 (1.53)			3.36
		sub-adult	40	0.09[a] (0–1.24)	8.56[a] (7.44–9.82)	4.63 (2.04)	1.34	9.2	
Eosinophil count	10^{9} l^{-1}	adult	155	0.04[b] (0–0.07)	1.39[b] (1.28–1.44)	0.56 (0.36)			0.51
		sub-adult	39	0[a] (0)	1.1[a] (0.9–1.28)	0.56 (0.36)	0	1.44	
Monocyte count	10^{9} l^{-1}	adult	155	0[b] (0)	0.43[b] (0.35–0.53)	0.11 (0.12)			0.07
		sub-adult	40	0[b] (0)	0.66[b] (0.65–0.8)	0.19 (0.19)	0	0.66	0.15
Basophil count	10^{9} l^{-1}	adult	155	0[b,d] (0–0.08)	0.08[b,d] (0.07–0.1)	0.01 (0.02)			0
		sub-adult	40	0[b,d] (0)	0.09[b,d] (0.09–0.13)	0.01 (0.03)	0	0.09	0
HGB	g l^{-1}	adult	175	98.8[b] (92–106)	164[b] (158–170)	129.58 (16.4)			128
		sub-adult	51	88.28[a] (80.99–95.48)	162.52[a] (154.8–1713)	125.27 (18.27)			
HCT	l l^{-1}	adult	179	0.15[b] (0.1–0.16)	0.41[b] (0.39–0.43)	0.27 (0.07)			0.26
		sub-adult	54	0.12[a] (0.08–0.16)	0.47[a] (0.44–0.5)	0.29 (0.09)			
MCV	Fl	adult	178	61.82[b] (58.27–63.4)	108.35[b] (105.56–109.86)	92.77 (10.66)			95.23
		sub-adult	54	60.95[a] (54.91–65.65)	112.17[a] (106.59–116.52)	85.73 (12.42)			
MCH	pg	adult	179	29.94[b] (24.69–32.86)	75.62[b] (69.51–86.76)	47.21 (11.58)			45.4
		sub-adult	53	15.06[a] (10.28–19.02)	60.72[a] (56.44–65.15)	38.31 (11.22)			
MCHC	g l^{-1}	adult	180	352.13[b] (343.95–364.53)	793.64[b] (718.31–1156.86)	509.79 (131.72)			482.74
		sub-adult	54	315.47[b] (293.16–322.74)	1132.14[b] (984.29–1602.43)	457.46 (156.72)			418.9
PLT	10^{9} l^{-1}	adult	168	74.38[b] (58–94)	259.55[b] (238–318)	155.74 (43.97)			152
		sub-adult	51	11.43[a] (0–33.92)	278.87[a] (247.78–310.4)	154.12 (65.1)			
NRBC count	10^{9} l^{-1}	adult	45	0[b,d] (0)	0.16[b,d] (0.15–0.18)	0.04 (0.04)	0	0.16	0.02
		sub-adult	26	0[b,d] (0)	0.06[b,d] (0.06–0.07)	0.02 (0.02)	0	0.06	0.02
Albumin	g l^{-1}	adult	68	16.28[a] (13.99–18.55)	42.27[a] (40.23–44.48)	28.97 (6.46)			
		sub-adult	32	22.32[c] (19.88–24.75)	41.53[c] (39.09–43.96)	31.92 (4.9)	17	40.7	
TP	g l^{-1}	adult	31	59.14[a] (58.65–59.14)	80.99[a] (80.99–85.42)	67.14 (5.5)	59.14	80.99	
		sub-adult	26	48.75[c] (44.66–52.84)	77.85[c] (73.76–81.94)	63.3 (7.42)	45.7	73.88	
Globulin	g l^{-1}	adult	30	24.35[a] (21.85–26.66)	41.76[a] (39.98–43.79)	32.8 (4.15)	25.12	40.03	
		sub-adult	26	19.63[c] (16.74–22.53)	40.24[c] (37.34–43.14)	29.94 (5.26)	20.38	39.39	

RBC, red blood cell count; WBC, white blood cell count; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelets; NRBC, nucleated red blood cell count; TP, total protein; CI, confidence interval; SD, standard deviation.
[a] Calculated using robust methods.
[b] Calculated using nonparametric methods.
[c] Calculated using parametric methods.
[d] Range of observed values.

Table 4. Seasonal haematological, glucose and serum protein intervals for free ranging eastern grey kangaroos *Macropus giganteus* sampled from reference sites; Nelson Bay Golf Course, (NBGC), Darlington Park (DP), Heritage Park (HP) and Ainslie Majura Kangaroo Management Unit (KMU) (AM) from 2015 to 2019.

Parameter	Units	Season	n	Lower reference interval (CI)	Upper reference interval (CI)	Mean (SD)	Minimum	Maximum	Median
RBC count	10^{12} l^{-1}	summer	47	1.88[a] (1.68–2.04)	3.59[a] (3.43–3.76)	2.74 (0.41)			
		autumn	101	0.7[a] (0.27–1.1)	5.52[a] (5.08–5.99)	3.3 (1.21)			
		winter	63	1.43[a] (1.15–1.67)	4.82[a] (4.55–5.12)	3.13 (0.84)			
		spring	19			2.18			
WBC count	10^{9} l^{-1}	summer	49	2.66[a] (1.91–3.42)	10.97[a] (10.22–11.84)	6.84 (2.04)			
		autumn	101	0.01[a] (0–0.9)	13.74[a] (12.69–14.8)	7.07 (3.44)			
		winter	61	3.21[a] (2.56–3.79)	9.95[a] (9.33–10.63)	6.72 (1.65)			
		spring	17			7.1			
Glucose	mmol l^{-1}	summer	49	1.69[a] (1.29–2.1)	5.53[a] (5.03–5.95)	3.72 (0.94)			
		autumn	44	0.25[a] (0–1.13)	7.02[a] (6.18–7.97)	3.87 (1.65)			
		winter	23	1.7[c] (1.33–2.07)	4.2[c] (3.83–4.58)	2.95 (0.64)	1.7	4.3	
		spring	17			3.86			
Neutrophil count	10^{9} l^{-1}	summer	47	0.91[b] (0.58–0.92)	5.66[b] (5.61–6.2)	2.76 (1.23)			2.39
		autumn	94	0[a] (0)	5.08[a] (4.65–5.54)	2.41 (1.39)			
		winter	49	0[a] (0–0.47)	4.62[a] (4.01–5.33)	2.44 (1.15)			
		spring	13			2.4			
Lymphocyte count	10^{9} l^{-1}	summer	47	1.03[a] (0.62–1.55)	5.49[a] (5.05–5.98)	3.28 (1.09)			
		autumn	90	0.81[b] (0.32–0.83)	8.74[b] (8.27–9.29)	4.17 (2.02)			3.76
		winter	49	1.54[b] (1.1–1.58)	7.26[b] (6.91–8.76)	3.5 (1.29)			3.17
		spring	11			3.77			
Eosinophil count	10^{9} l^{-1}	summer	44	0[a] (0–0.03)	1.09[a] (0.96–1.23)	0.52 (0.29)			
		autumn	91	0.02[b] (0–0.04)	1.37[b] (1.34–1.45)	0.54 (0.38)			0.44
		winter	47	0[a] (0)	1.34[a] (1.17–1.54)	0.58 (0.38)			
		spring	12			0.55			
Monocyte count	10^{9} l^{-1}	summer	45	0[b] (0)	0.45[b] (0.44–0.56)	0.11 (0.12)			0.08
		autumn	90	0[b] (0)	0.53[b] (0.34–0.62)	0.13 (0.16)			0.07
		winter	47	0[b] (0)	0.22[b] (0.22–0.23)	0.07 (0.08)			0.04
		spring	12			0.34			
Basophil count	10^{9} l^{-1}	summer	42	0[b,d] (0)	0.01[b,d] (0.01–0.03)	0 (0)			0
		autumn	90	0[b,d] (0)	0.05[b,d] (0.02–0.07)	0.01 (0.02)			0
		winter	45	0[b,d] (0)	0.1[b,d] (0.1–0.11)	0.01 (0.03)			0
		spring	12			0.02			
HGB	g l^{-1}	summer	46	93.26[a] (86.92–99.17)	153.07[a] (146.45–159.62)	124.04 (14.64)			
		autumn	99	94.04[a] (89.69–97.95)	154.86[a] (150.11–159.52)	125.39 (15.18)			
		winter	62	104.23[a] (98.91–109.54)	169.27[a] (164.13–174.2)	136.55 (16.01)			
		spring	16			123.5			
HCT	l l^{-1}	summer	49	0.12[a] (0.1–0.14)	0.33[a] (0.3–0.35)	0.23 (0.05)			
		autumn	102	0.14[a] (0.12–0.16)	0.44[a] (0.42–0.46)	0.29 (0.07)			
		winter	63	0.16[a] (0.13–0.18)	0.44[a] (0.42–0.47)	0.3 (0.07)			
		spring	19			0.2			
MCV	Fl	summer	49	62.2[a] (56.57–69.76)	111.86[a] (108.31–115.94)	84.15 (12.19)			
		autumn	101	62.48[b] (60.29–64.45)	108.33[b] (107.66–110.93)	91.69 (11.71)			94.42
		winter	63	81.61[a] (78.27–84.87)	112.1[a] (109.61–114.74)	95.89 (7.53)			
		spring	19			90.4			

(Continued)

Table 4. Continued.

Parameter	Units	Season	n	Lower reference interval (CI)	Upper reference interval (CI)	Mean (SD)	Minimum	Maximum	Median
MCH	pg	summer	49	29.18[a] (26.25–31.7)	62.6[a] (59.41–66.72)	46.34 (8.02)			
		autumn	102	21.08[b] (19.89–21.68)	75.57[b] (75.03–80.81)	42.5 (14.1)			39.21
		winter	64	28.48[c] (25.49–31.48)	61.88[c] (58.88–64.87)	45.18 (8.52)			
		spring	19			57.99			
MCHC	g l^{-1}	summer	49	341.79[a] (298.79–379.42)	770.6[a] (728.26–816.97)	558.82 (105.27)			
		autumn	102	331.31[b] (320.66–354.43)	714.02[b] (628.03–755.66)	454.42 (112.24)			404.3
		winter	64	378.46[b] (373.17–382.62)	686.92[b] (683.36–755.6)	469.74 (81.05)			441.77
		spring	19			666.8			
PLT	10^9 l^{-1}	summer	44	95[b] (77.75–96)	311.5[b] (305–376.5)	159.77 (44.63)			150
		autumn	100	36.62[a] (20.48–52.55)	265.68[a] (248.24–284.19)	154.98 (57.35)			
		winter	57	73.23[a] (60.83–86.23)	209.94[a] (196.2–224.86)	141.3 (33.79)			
		spring	19			148.47			
NRBC count	10^9 l^{-1}	summer	17			0.04			0.02
		autumn	42	0[b,d] (0)	0.08[b,d] (0.08–0.1)	0.02 (0.02)			0.02
		winter	4			0.01			
		spring	6			0.01			
Albumin	g l^{-1}	summer	28	23.73[c] (21.1–26.35)	43.1[c] (40.47–45.72)	33.41 (4.94)	25	41.06	
		autumn	28	21.63[c] (18.95–24.31)	43.39[c] (38.71–44.07)	31.51 (5.04)	16	39	
		winter	33	16.01c (13.77–18.25)	33.94[c] (31.7–36.17)	24.97 (4.57)	16	34.66	
		spring	8			32.72			
TP	g l^{-1}	summer	25	53.68[c] (50.19–57.17)	78.01[c] (74.52–81.5)	65.85 (6.21)	50.57	76.62	
		autumn	16			65.25			
		winter	6			65.17			
		spring	8			65.34			
Globulin	g l^{-1}	summer	27	21.71[c] (18.88–24.53)	42.17[c] (39.34–44.99)	31.94 (5.22)	20.38	40.03	
		autumn	18			32.04 (32.38)			
		winter	6			31.4			
		spring	6			30.82			

RBC, red blood cell count; WBC, white blood cell count; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelets; NRBC, nucleated red blood cell count; TP, total protein; CI, confidence interval; SD, standard deviation.
[a] Calculated using robust methods.
[b] Calculated using nonparametric methods.
[c] Calculated using parametric methods.
[d] Range of observed values.

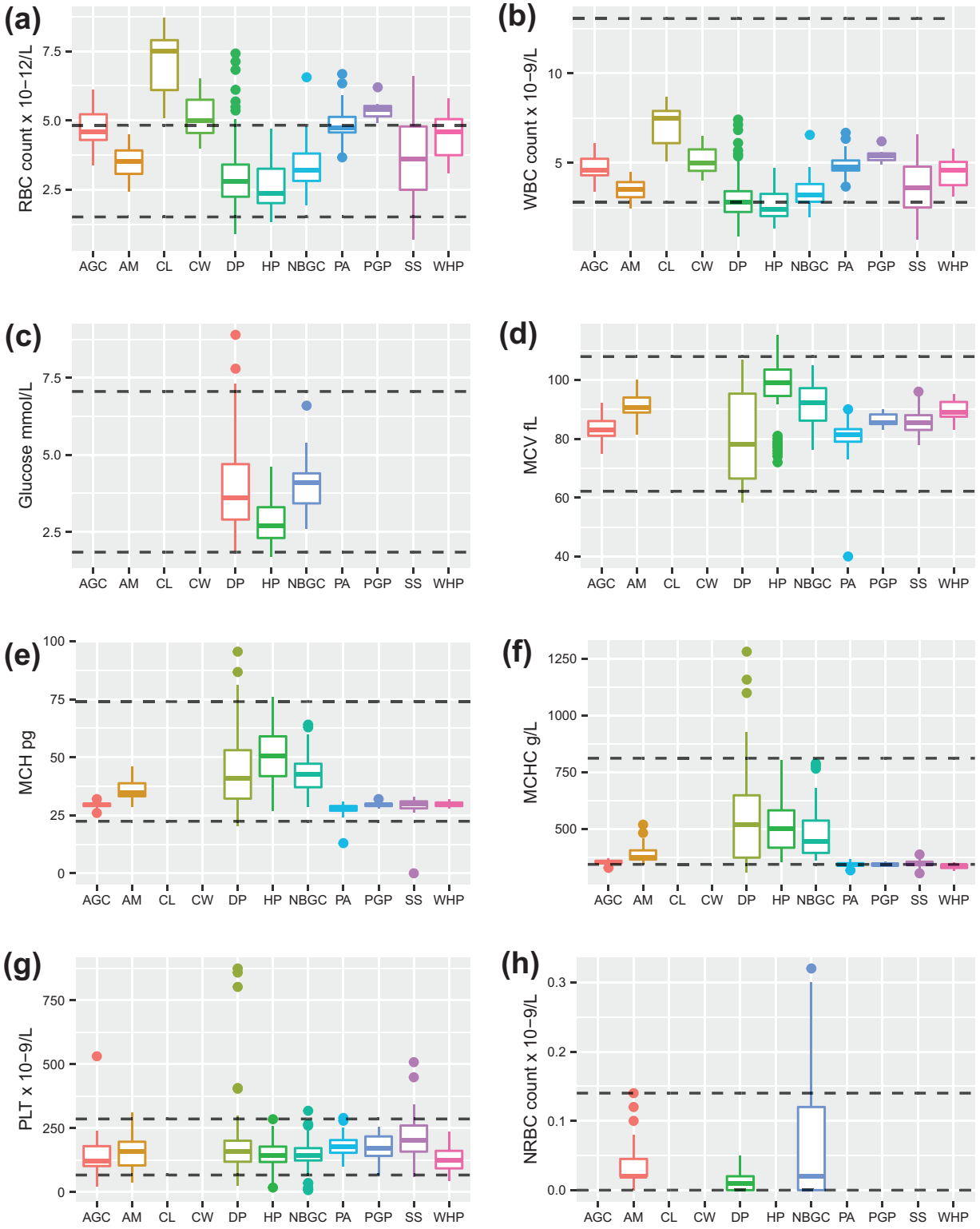

Figure 2. Box and whisker plots of site specific mean haematological, glucose and protein values for eastern grey kangaroos *Macropus giganteus* from 11 populations; Nelson Bay Golf Course, (NBGC), Darlington Park (DP), Heritage Park (HP), Ainslie Majura Kangaroo Management Unit (KMU) (AM), Anglesea Golf Course (AGC), Serendip Sanctuary (SS), Woodlands Historic Park (WHP), Portland Aluminium (PA), Cowan (CW) and Calga (CL). Box plots display the median, with the lower and upper limits of the box corresponding to the 25th and 75th percentiles. The upper whisker extends to the largest value no further than 1.5 times the interquartile range. The lower whisker extends to the smallest value at most 1.5 times the interquartile range. Data beyond the end of the whiskers are outliers and are plotted individually. Dashed lines indicate upper and lower reference intervals (RI) as calculated from the reference sites. (a) red blood cell count (RBC), (b) white blood cell count (WBC), (c) glucose, (d) mean corpuscular volume (MCV), (e) mean corpuscular haemoglobin (MCH), (f) mean corpuscular haemoglobin concentration (MCHC), (g) platelets (PLT), (h) nucleated red blood cell count (NRBC), (i) neutrophil count, (j) lymphocyte count, (k) eosinophil count, (l) monocyte count, (m) basophil count , (n) albumin, (o) total protein (TP), (p) globulin, (q) haemoglobin (HGB), (r) haematocrit (HCT).

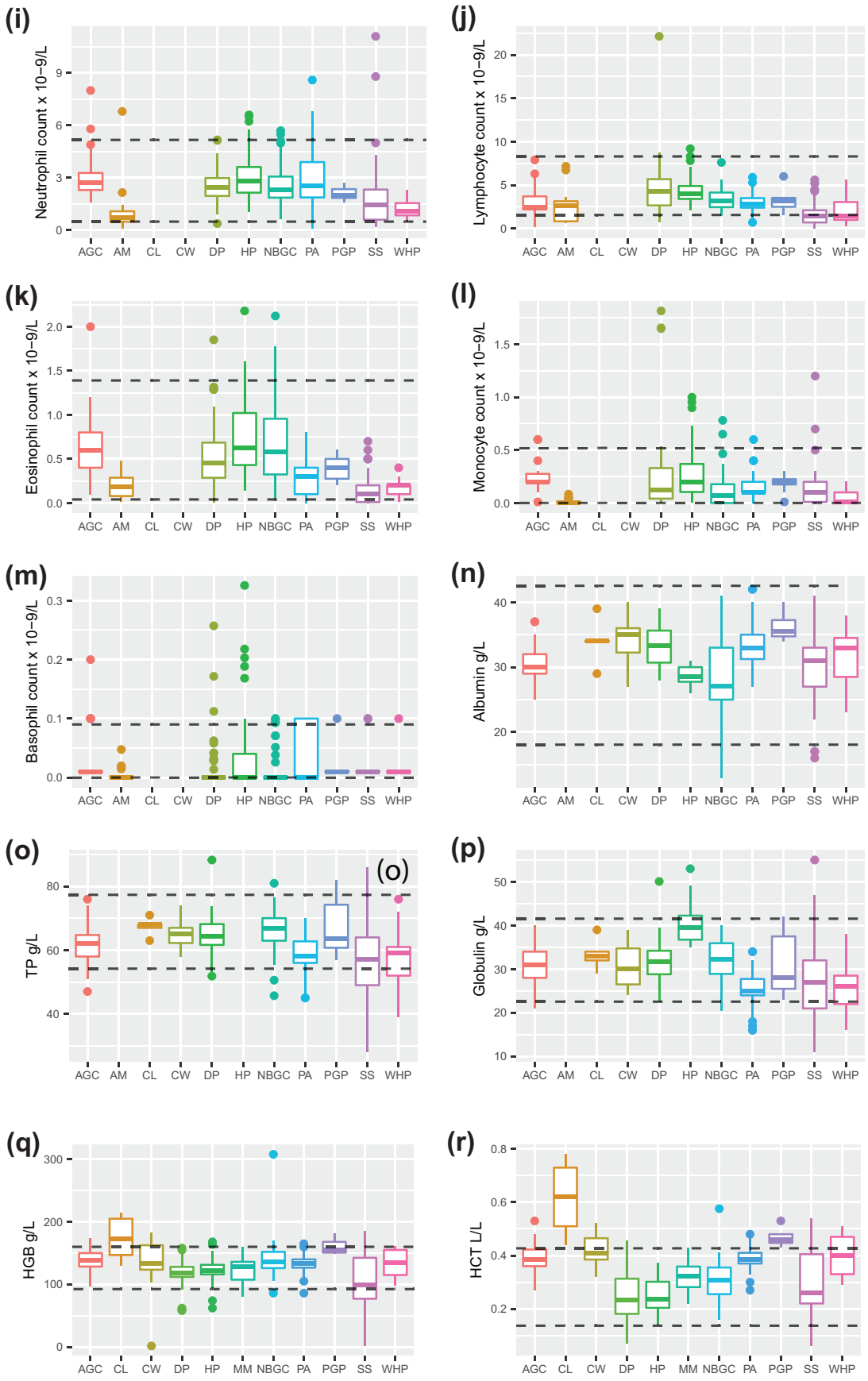

Figure 2. Continued

Table 5. Mean and 95% confidence intervals of effect sizes (ES, Cohen's *d* and eta squared (η^2)) of the factors sex, season and sexual maturity on haematological and serum protein values. Significance values from one-way analysis of variance (ANOVA) for effects of sex, season and sexual maturity for each parameter for free ranging eastern grey kangaroos *Macropus giganteus* sampled from reference sites; Nelson Bay Golf Course, (NBGC), Darlington Park (DP), Heritage Park (HP) and Ainslie Majura Kangaroo Management Unit (KMU) (AM) from 2015 to 2019.

	Sex					Season					Maturity				
Parameter	ES	ED	Low CI	Upper CI	p	ES	ED	Low CI	Upper CI	p	ES	ED	Low CI	Upper CI	p
RBC count	−0.27	small	−0.54	−0.01	**0.02**	0.14[a]	large	0.07	0.23	**<0.001**	−0.49	small	−0.8	−0.18	**0.02**
WBC count	0.04	negligible	−0.22	0.3	0.75	0[a]	none	0	0	0.98	−0.23	small	−0.54	0.07	0.1
Glucose	−0.75	medium	−1.11	−0.4	**<0.001**	0.05[a]	medium	0	0.14	**0.04**	−0.76	medium	−1.16	−0.36	**<0.01**
Neutrophil count	−0.08	negligible	−0.36	0.2	0.56	0.02[a]	small	0	0.06	0.3	0.49	small	0.14	0.83	**<0.01**
Lymphocyte count	0.07	negligible	−0.21	0.35	0.6	0.03[a]	small	0	0.08	0.12	−0.65	medium	−1	−0.31	**<0.001**
Eosinophil count	−0.19	negligible	−0.87	0.49	0.51[b]	0[a]	none	0	0.01	0.46[b]	−0.13	negligible	−0.99	0.73	0.41[b]
Monocyte count	−0.23	small	−0.5	0.05	0.1	0.07[a]	medium	0	0.13	**<0.01**	−0.25	small	−0.59	0.1	0.31
Basophil count	0.2	small	−0.08	0.48	0.75[b]	0.03[a]	small	0	0.08	0.06[b]	−0.12	negligible	−0.46	0.22	0.41[b]
HGB	−0.09	negligible	−0.35	0.17	0.47	0.09[a]	medium	0.02	0.15	**<0.001**	0.27	small	−0.03	0.58	**<0.01**
HCT	−0.16	negligible	−0.42	0.1	0.16	0.24[a]	large	0.14	0.32	**<0.001**	−0.25	small	−0.55	0.06	0.93
MCV	0.29	small	0.03	0.55	**<0.001**[b]	0.11[a]	large	0.05	0.2	**<0.001**[b]	0.62	medium	0.31	0.94	**<0.001**[b]
MCH	0.28	small	0.02	0.55	**0.02**	0.14[a]	large	0.07	0.23	**<0.001**	0.74	medium	0.42	1.05	**<0.001**
MCHC	0.14	negligible	−0.12	0.4	0.23	0.25[a]	large	0.15	0.33	**<0.001**	0.52	medium	0.21	0.83	**0.03**
PLT	0	none	−0.26	0.26	0.98	0.05[a]	medium	0.01	0.11	**0.01**	0.12	negligible	−0.19	0.43	0.97
NRBC count	−0.26	small	−0.74	0.22	0.28	0.08[a]	medium	0	0.2	0.08	0.1	negligible	−0.38	0.58	0.91
Albumin	−0.1	negligible	−0.5	0.3	0.51	0.34[a]	large	0.21	0.48	**<0.001**	−0.49	small	−0.92	−0.06	**<0.001**
TP	0.04	negligible	−0.5	0.59	0.87	0[a]	none	0		0.2	0.58	medium	0.04	1.12	**<0.01**
Globulin	−0.09	negligible	−0.64	0.46	0.72	0[a]	none	0	0.01	0.96	0.66	medium	0.11	1.21	**<0.01**

RBC, red blood cell count; WBC, white blood cell count; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelets; NRBC, nucleated red blood cell count; TP, total protein; ES, effect size; ED, effect description; CI, confidence intervals; p, significance.
Bold indicates significant effect of <0.05.
[a] ES calculated using eta squared (η^2).
[b] Significance values from Kruskal–Wallis (KW) test (non-Gaussian distribution).

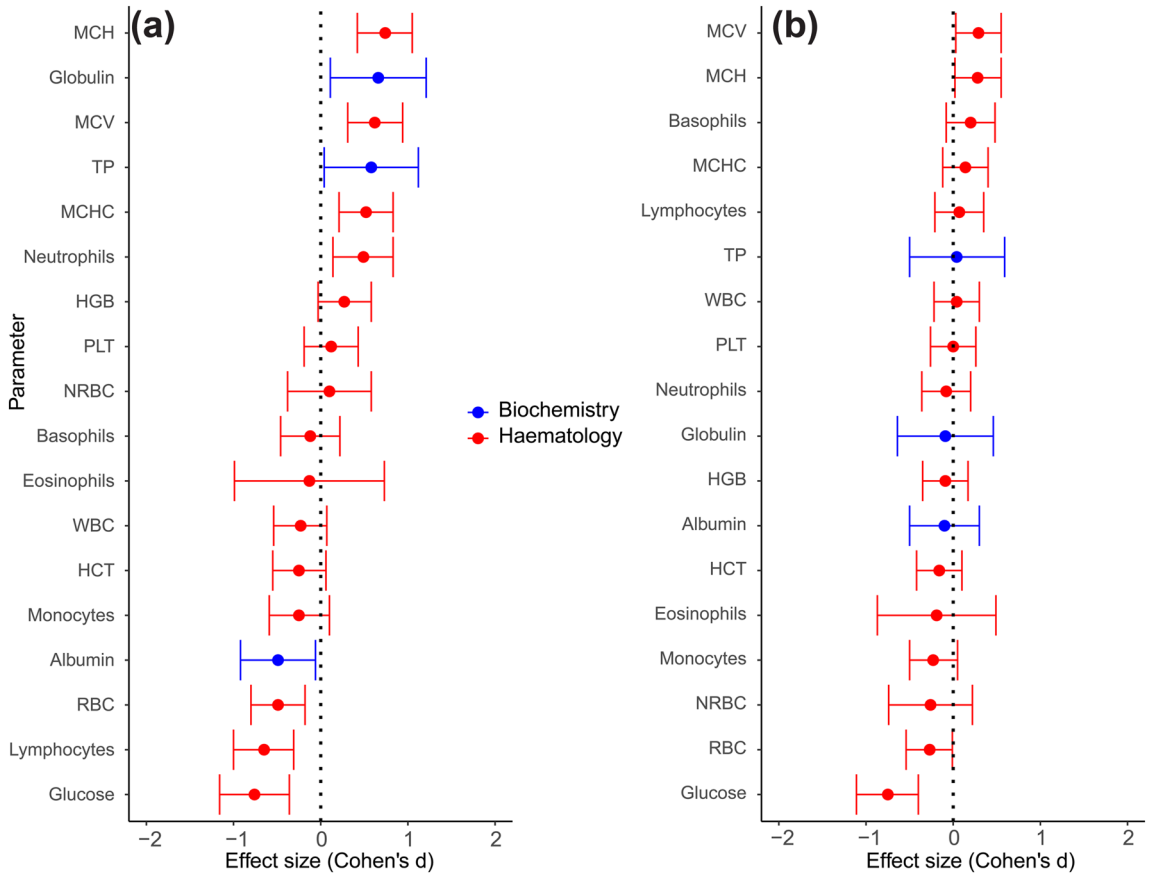

Figure 3. Forest plot of the effect size (Cohen's *d* and 95% confidence intervals) of (a) sexual maturity and (b) sex on haematological, glucose and serum protein values from the reference population (Nelson Bay Golf Course, NBGC; Darlington Park, DP; Heritage Park, HP; Ainslie Majura Kangaroo Management Unit (KMU) (AM)) of wild eastern grey kangaroos *Macropus giganteus*. ES of 0.2, 0.5 and 0.8 indicate small, medium and large effect sizes respectively (Cohen 1988). (a) Negative Cohen's *d* indicates adults are lower than sub-adults for that parameter. Positive Cohen's *d* indicates adults are higher than sub-adults for that parameter. (b) Negative Cohen's *d* indicates females are lower than males for that parameter. Positive Cohen's *d* indicates females are higher than males for that parameter.

on WBC, eosinophil counts, TP and globulin concentrations. Based on these results, RIs and mean blood parameters were partitioned by season (Table 4).

Random forest model of factors influencing parameters of health

Abiotic factors were the most important drivers of RF predictions of haematological, glucose and serum protein values across all 11 sites (Fig. 5). Site was consistently the most important predictor, followed by rainfall, temperature, season, laboratory, maturity and sex (ranked 0). RBC count and derived parameters (HCT, MCV, MCH and MCHC) were best explained by biotic and abiotic factors compared to all other parameters, as indicated by a greater PVE (Supplementary material Appendix 1 Table A2). All models were significant ($p < 0.05$–0.001) except for NRBC count (Supplementary material Appendix 1 Table A2). HGB, MCHC and PLT count have large RMSEs indicating a high error rate within the model (Supplementary material Appendix 1 Table A2). PDPs were generated for RBC count and derived parameters (HCT, MCV, MCH and MCHC) as the PVE was greater than 50. PDPs showed a pattern of decreasing RBC count with increasing rainfall up to 100 mm, then an increase in RBC count. Increasing rainfall had a decreasing effect on HCT and an increasing effect on MCV, MCH and MCHC. Increasing temperature also had a decreasing effect on RBC count to 22°C, where RBC counts then increased. There were no simple patterns for the effect of temperature on HCT, MCV, MCH and MCHC.

Discussion

This study establishes RIs for several parameters of health for free ranging eastern grey kangaroos and investigated the significance of abiotic and biotic factors influencing these values. This study is novel as it develops the first RI for haematological, glucose and serum protein parameters for free ranging eastern grey kangaroos and characterises these values across multiple populations throughout a large portion of the species geographical range.

Of the biotic factors considered, sex had a negligible effect on most of the parameters and was ranked zero in importance from the RF model across sites. This is consistent with previous investigations that found no effect of sex on WBC counts in eastern grey kangaroos and agile wallabies *Notamacropus agilis* (Presidente 1978, Stirrat 2003). However, glu-

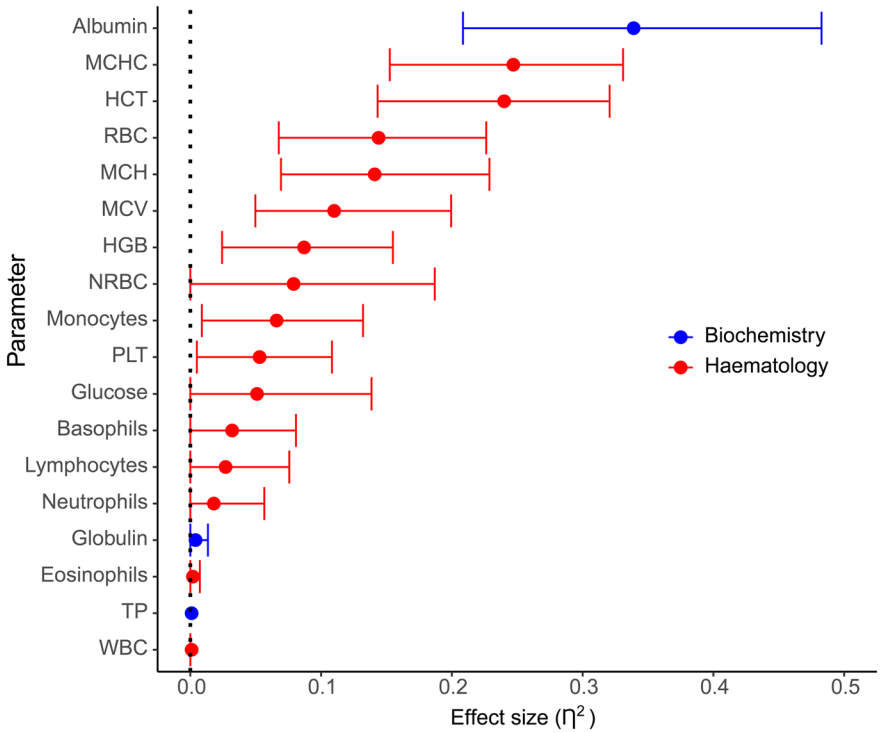

Figure 4. Forest plot of the effect size (ES, eta squared (η^2) and 95% confidence intervals) of season on haematological, glucose and serum protein values from the reference population (Nelson Bay Golf Course, NBGC; Darlington Park, DP; Heritage Park, HP; Ainslie Majura Kangaroo Management Unit (KMU) (AM)) of free ranging eastern grey kangaroos *Macropus giganteus*. ES of 0.01, 0.06 and 0.14 indicate small, medium and large effect sizes respectively (Maher et al. 2013).

cose concentrations were lower in female compared to male eastern grey kangaroos, as reported in this species (Green-Barber et al. 2018) and in angora rabbits *Oryctolagus cuniculus* (Cetin et al. 2009). In addition, the sex-specific glucose RI for female kangaroos is much wider than for males, demonstrating that normal female glucose levels fluctuate more than in males. This is an interesting finding, as maternal glucose has been correlated with offspring sex determination (Helle et al. 2008) and there is evidence of condition-dependent sex bias in young kangaroos (Gall-Payne et al. 2015). Higher glucose concentration in males could also be attributed to a stress response (Peck et al. 2015). It is well known amongst social, sexually-dimorphic animals that maintaining social dominance is a physiologically demanding activity (Creel 2005), thereby enhancing circulating glucose levels in response to glucocorticoids. We did not consider the effect of female reproductive status on blood parameters because the effects of pregnancy and lactation are unclear in other species (Harvey et al. 1994, Alonso et al. 1997, Harewood et al. 2000), and sex was ranked poorly in our RF model. However, we recognise that the long lactation period in kangaroos could affect some haematological, glucose and serum protein concentrations.

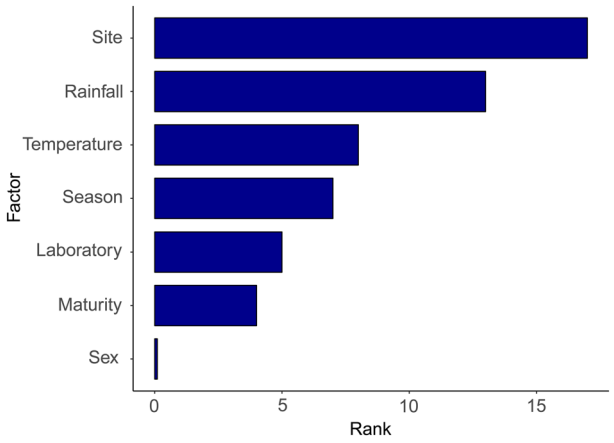

Figure 5. Importance rank of biotic and abiotic factors driving random forest (RF) predictions of haematological, glucose and serum protein parameters from 11 eastern grey kangaroo *Macropus giganteus* populations; Nelson Bay Golf Course, (NBGC), Darlington Park (DP), Heritage Park (HP), Ainslie Majura Kangaroo Management Unit (KMU) (AM), Anglesea Golf Course (AGC), Serendip Sanctuary (SS), Woodlands Historic Park (WHP), Portland Aluminium (PA), Cowan (CW) and Calga (CL).

The immune system undergoes ontogenesis (Sidman et al. 1987), with many haematological and some serum protein parameters increasing with age (Fancourt and Nicol 2019). Consistent with this, maturity was partitioned in our RI because it was determined as moderately important in our RF model, with adults having significantly higher MCV, MCH, MCHC, TP and globulin concentrations than subadults. Partitioning based on maturity also satisfied statistical criteria. Similar findings have been demonstrated in other marsupial species. For example, TP increases with age in the tammar wallaby *Notamacropus eugenii*, short-eared mountain possum *Trichosurus caninus* and southern hairy-nosed wombat *Lasiorhinus latifrons* (Barnett et al. 1979, McKenzie et al. 2002, Woolford et al. 2020); and in small mammals, RBC values are higher in juveniles when compared

with adults (Sealander 1964). The significantly lower values for glucose concentrations and lymphocyte counts in adults is likely related to decreased glucose metabolism and the output of T lymphocytes as the immune response declines with age (Clot et al. 1978, Defronzo 1981, Linton and Dorshkind 2004). Given the observed maturity differences in blood parameters, it is important that sub-adult individuals are clinically evaluated with RIs specific to this age cohort.

While the purpose of this study was to develop haematological, glucose and serum protein RIs for kangaroos, the effect of analytical laboratory on these parameters was also assessed. Analysis of blood data by three different commercial laboratories showed that laboratory was more important than biological factors (sex and sexual maturity) but less important than environmental factors (site, rainfall and temperature) on haematological, glucose and protein parameters. This has implications for the interpretation of health parameters depending on the scale of the investigation. For assessing population health across sites or seasonal changes in blood parameters, laboratory is of lesser importance as environmental factors have the largest impact. However, when comparing individuals based on biological factors, differences between laboratories could mask underlying biological differences. There are many potential sources of difference among commercial laboratories: instrumentation, laboratory environmental conditions, quality control (calibration and standard operating procedures) and technician variation in manual determinations, for example, of differential WBC counts (Flatland et al. 2010). As a result of these differences, when monitoring intra-individual and biological factors it is recommended that the same laboratory is used.

Site was consistently the most important predictor of haematological, glucose and serum protein parameters, most likely because these parameters are highly sensitive to season, local environmental cues, intrinsic population characteristics, genetics and other non-climatic variables (Argente et al. 2014). The consistently large relative importance of rainfall compared to temperature and season suggests that from all factors modelled, rainfall drives the variation among sites. Due to limitations within our dataset, we cannot account for other sources of variation that might contribute to the importance of site; such as habitat type, food and water availability. Our PDPs show that for some haematological parameters, rainfall and temperature have a strong influence. When there is < 100 mm of rainfall in the month prior to sampling, the RBC count and HCT are high, likely due to increased water loss or decreased water intake and subsequent haemoconcentration (Clark 2004). RBC count then begins to decline as rainfall increases, until 100 mm. The relationship in RBC count reverses above 100 mm of rainfall, where RBC counts begin to increase. A similar pattern was found for temperature: there is an initial decrease in RBC count in cooler temperatures, then above 22°C the RBC count increases. These highly seasonal effects on RBC counts have been shown in several species of dasyurids and echidnas (Andersen et al. 2000, Clark 2004) and in macropods. In wallaroos *Osphranter robustus*, rainfall was shown to influence the nutrient content of plants, particularly proteins, which is correlated with increased RBC parameters (Ealey and Main 1967). MCV, MCH and MCHC were also shown to increase in response to increasing rainfall. Seasonal variation was evident in most of the haematological, glucose and serum protein parameters analysed, highlighting the importance of establishing season-specific RIs. If haematological parameters are being used to evaluate changes in condition of a population over time, it is recommended that samples are collected at the same time of year, to coincide with similar weather patterns. Specifically, RBC, HCT, MCV, MCH, MCHC, albumin, glucose, monocyte count, HGB, PLT and NRBC count are the parameters most affected by season. These effects are likely to become more pronounced as Australia's climate changes. This study was conducted through an extended period of drought in eastern Australia (Bureau of Meteorology 2018). Australia's climate is predicted to become warmer and drier over the next decade, with rainfall occurring in increasingly isolated and sporadic events (Bureau of Meteorology 2018). Our results suggest that this changing climate will likely impact these indicators of kangaroo health.

As site was identified as the most important predictor of changes in haematological, glucose and serum protein values in this study, blood samples were analysed from a range of unique site-specific populations which varied in population density, presence of endemic disease (for example high fluoride exposed animals, parasitism and oral necrobacilliosis), food availability (inadequate to abundant) and water resources (for example artificial water resources at golf courses). Variations in haematological, glucose and serum protein parameters, due to environmental, parasitic and biological factors have been described in many wild mammalian species (Lepitzki and Woolf 1991, Spencer and Speare 1992). It is likely that these intrinsic population-based factors, in addition to environmental factors, are responsible for differences in haematological, glucose and serum protein values. Environmental factors such as rainfall, temperature and their seasonal changes, directly influence parasite load, reproductive stressors and nutrient supply, through food availability and quality (Chaplin and White 1972, Ellis et al. 1977, Magona and Musisi 2002). These factors influence an individual's haematological values accordingly. For example, increased numbers of haematophagus gastrointestinal nematodes can result in seasonal variation in RBC counts, corresponding to seasonal variation in parasite burden (Pacioni et al. 2013).

The RIs were developed from sampling chemically restrained kangaroos from the lateral caudal vein. However, animals from AM ($n = 28$) were bled within 30 minutes post-mortem by direct cardiac venipuncture. Differences in haematological, glucose and serum protein parameters due to post-mortem and venipuncture site have been reported (Maceda-Veiga et al. 2015). Specifically, MCV may increase immediately after death, as water temporarily moves from the extracellular to the intracellular fluid compartment (Hodgkinson and Hambleton 1969). HCT and glucose concentration also show variability between white tailed deer *Odocoileus virginianus* bled immediately after death versus animals bled 30 min later (Wesson Iii et al. 1979). However, comparisons between AM and sites where chemical restraint was used show that MCV and PCV were not significantly different. For this reason, it was considered appropriate to include data from AM in the RI.

Most haematological, glucose and serum protein parameters determined from each site in this study had trends consistent with the developed RI. Most values fell within the RI or overlapped the upper or lower limits of the range; however, some key differences were seen. Previous reports in macropods have indicated that kangaroos have higher neutrophil compared to lymphocyte counts (ISIS 2002, Vogelnest and Portas 2008, Green-Barber et al. 2018). In this study, except for kangaroos sampled at two sites (AGC and SS) kangaroos had higher lymphocyte compared to neutrophil counts, consistent with a single report from four individual kangaroos (Spencer et al. unpubl., cited in Clark 2004) and large grazing ruminants (Jones and Allison 2007). Higher neutrophil counts at SS could be evidence of inflammatory demand (Maceda-Veiga et al. 2015) as a result of the extremely high prevalence of lumpy jaw at this site or a corticosteroid-mediated stress-response in unhealthy individuals. Alternatively, a higher lymphocyte count could indicate greater immunostimulation at most of the sites sampled or an adrenalin-mediated physiological lymphocytosis (Clark 2004). This is a significant finding when interpreting blood values for kangaroos.

Some site-specific differences in haematological, glucose and serum protein parameters could be attributed to captivity. For example, at CL and CW animals receive supplementary food, whilst CW also received intestinal parasite treatment. Both sites had higher RBC counts and HCT, and kangaroos sampled at CL had higher HGB concentrations. Captive diets high in protein are beneficial to production of RBCs (Amin et al. 2007), while elimination of internal and external parasitism would reduce the likelihood of anaemia (reduced HCT, RBC count and HGB) and protein loss (Blackburn et al. 1992). Previously reported mean RBC and HCT counts from captive colonies are also higher than the RI reported (Spencer et al. unpubl., cited in (Clark 2004), ISIS 2002). The variation in these parameters of health could also be attributed to site-specific influences, such as rainfall and temperature, as environmental factors were important predictors of haematological, glucose and serum protein parameters in our RF models.

Our findings have informed some guidelines for assessing kangaroo health. Managers should aim to capture a representative sample of the population. A targeted approach should be taken where managers survey populations within a given timeframe and analyse samples as a batch at one laboratory, rather than relying on opportunistic sampling of individuals at different time points and using different laboratories. Health surveillance should also be conducted within a season, and maturity specific RIs used for the relevant subgroups. Most analytical laboratories offer a 'profile' of tests which include most of the haematological and serum protein parameters described in this study. The RI developed in this study can be used as an aid to evaluate the health status of captive populations of eastern grey kangaroos given their species specificity. However, caution needs to be employed in the interpretation of individual results due to the inherent differences between captive and wild animals such as levels of parasitism and nutritional differences. For example, in this study, captive populations (CL and CW) had higher RBC, HCT and HGB counts compared to wild populations. For captive populations of kangaroos in countries with extreme climatic variability, consultation with the season specific RI could be advantageous. Ideally, a RI for captive individuals comprising greater than 120 healthy individuals, would be considered the most appropriate RI for health evaluation of captive individuals (Friedrichs et al. 2012).

Conclusion

This study highlights the importance of both biotic and abiotic factors on the establishment of RIs for parameters of health. Use of these RIs in differing contexts therefore needs to be undertaken with caution. In this study, site and specific environmental factors inherent in different sampling locations were shown to be the most important factors affecting these parameters of health in eastern grey kangaroos. Because Australia is becoming increasingly hotter and drier, health parameters in kangaroos will likely be increasingly affected by rapid changes in climate. There is an imperative to study more populations of kangaroos in poor condition, to discern the role of abiotic factors compared to other causes of disease. Additionally, consideration of the effect of season on haematological, glucose and serum protein values, timing of sample collection throughout the year should be considered for meaningful comparisons among individuals, sites or time points. Importantly, attempting to compare samples from markedly different sites, samples analysed at different laboratories, or samples from populations with different age structures and disease status could mean that important fluctuations are masked.

Based on the findings in this study, it is recommended that the species-level RI be used with caution, and that maturity-specific RIs established in this study be used preferentially to inform clinical evaluation of eastern grey kangaroos. We also recommend consulting site-specific population means, when specific site attributes are of interest; for example, when population density is high, for captive populations or for populations with endemic disease. Understanding the plethora of factors that can influence haematological and serum protein values will improve the utility of developed RIs, providing wildlife managers and veterinarians with a robust tool to assess population health and improve the management and welfare standards for eastern grey kangaroos in Australia.

Acknowledgements – We would like to thank all the field volunteers who contributed to data collection from all sites. We especially thank Kangaroo Phil Murray for his dedication to kangaroo research. Thank you to Dr Holly Cope for invaluable assistance in the field and to staff past and present at VPDS. We would like to thank Nelson Bay Golf Course, NRMA Darlington Beach Holiday Park and the Coffs Harbour City Council for funding and support. We thank Jodie Gould, Lora Harker and Paul Pitman of Portland Aluminium for technical and practical assistance. Thank you to staff at Anglesea Golf Club, Serendip Sanctuary, Plenty Gorge Parklands and Woodlands Historic Park for facilitating research at their sites. We are grateful to Dr Sarah Garnick, and many other volunteers for assistance with fieldwork.

Funding – The studies were funded by the Australian Research Council Linkage Project (LP0560344), Holsworth Wildlife Research Endowment and Parks Victoria's Research Partners Panel.

Author contributions – All authors contributed to either sample collection, data analysis or the writing and review of the manuscript.
Conflicts of interest – There are no conflicts of interests to declare.
Permits – This work was conducted with Animal ethics approval from the University of Sydney, permit number: 2016/1062 and 2015/917. Animal collection was performed under relevant permits from all NSW, Victoria and ACT governments (scientific license number: SL100961 and SL102148).

References

Alonso, A. J. et al. 1997. The effects of age and reproductive status on serum and blood parameters in Merino breed sheep. – J. Vet. Med. Ser. A 44: 223–231.

Amin, A. S. et al. 2007. Seasonal variation in blood constituents of one-humped camel (*Camelus dromedarius*). – Pak. J. Biol. Sci 10: 1250–1256.

Andersen, N. A. et al. 2000. The effects of sex, season and hibernation on haematology and blood viscosity of free-ranging echidnas (*Tachyglossus aculeatus*). – Can. J. Zool. 78: 174–181.

Argente, M. J. et al. 2014. Effect of selection for residual variance of litter size on hematology parameters as immunology indicators in rabbits. – In: Proc. 10th World Congress on genetics applied to livestock production, Vancouver.

Banks, P. B. et al. 2000. Predation by red foxes limits recruitment in populations of eastern grey kangaroos. – Austral Ecol. 25: 283–291.

Barnett, J. L. et al. 1979. Blood parameters in natural populations of *Trichosurus* species (Marsupialia: Phalangeridae). I. Age, sex and seasonal variation in *T. caninus* and *T. vulpecula*. II. Influence of habitat and population strategies of *T. caninus* and *T. vulpecula*. – Aust. J. Zool. 27: 913–926.

Bureau of Meteorology 2018. State of the climate. – <www.bom.gov.au/state-of-the-climate>, accessed 30 Nov 2019.

Bureau of Meteorology 2020. Climate data online. – <www.bom.gov.au/climate/data>, accessed 30 Sep 2019.

Blackburn, H. D. et al. 1992. Interaction of parasitism and nutrition in goats: effects on haematological parameters, correlations and other statistical associations. – Vet. Parasitol. 44: 183–197.

Borland, D. et al. 2012. Oral necrobacillosis ('lumpy jaw') in a free-ranging population of eastern grey kangaroos (*Macropus giganteus*) in Victoria. – Aust. Mammal. 34: 29–35.

Breiman, L. 2001. Random forests. – Mach. Learn. 45: 5–32.

Cetin, N. et al. 2009. Effects of sex, pregnancy and season on some haematological and biochemical blood values in angora rabbits. – Scan. J. Lab. Anim. Sci. 36: 155–162.

Chaplin, R. E. and White, R. W. G. 1972. The influence of age and season on the activity of the testes and epididymides of the fallow deer, *Dama dama*. – Reproduction 30: 361–369.

Clark, P. 2004. Haematology of australian mammals. – CSIRO Publishing.

Clot, J. et al. 1978. Age-dependent changes of human blood lymphocyte subpopulations. – J. Clin. Exp. Immunol. 32: 346–351.

Cohen, J. 1988. Statistical power analysis for the behavioral sciences. – Lawrence Erlbaum Associates.

Coulson, G. 2001. Overabundant kangaroo populations in southeastern Australia. – In: Field, R. et al. (eds), Wildlife, land and people: priorities for the 21st Century. The Wildlife Society, Bethesda, MS, USA, pp. 238–242.

Coulson, G. M. 2008. Eastern grey kangaroo. – In: Dyck, S. V and Strahan, R. (eds), The mammals of Australia, 3rd edn. New Holland Publishers, Australia, pp. 335–338.

Coulson, G. et al. 2008. Long-term efficacy of levonorgestrel implants for fertility control of eastern grey kangaroos (*Macropus giganteus*). – Wildl. Res. 35: 520–524.

Creel, S. 2005. Dominance, aggression and glucocorticoid levels in social carnivores. – J. Mammal. 86: 255–264.

Cripps, J. et al. 2013. The efficacy of anthelmintic drugs against nematodes infecting free-ranging eastern grey kangaroos, *Macropus giganteus*. – J. Wildl. Dis. 49: 535–544.

Cripps, J. et al. 2014. Experimental manipulation reveals few subclinical impacts of a parasite community in juvenile kangaroos. – Int. J. Parasitol. Parasit. Wildl. 3: 88.

Cripps, J. K. et al. 2016. Anthelmintic treatment does not change foraging strategies of female eastern grey kangaroos, *Macropus giganteus*. – PLoS One 11: e0147384.

Cutler, D. R. et al. 2007. Random forests for classification in ecology. – Ecology 88: 2783–2792.

Death, C. et al. 2017. Skeletal fluorosis in marsupials: a comparison of bone lesions in six species from an Australian industrial site. – Sci. Total Environ. 584: 1198–1211.

Defronzo, R. A. 1981. Glucose intolerance and aging. – Diabetes Care 4: 493.

Descovich, K. et al. 2016. The eastern grey kangaroo: current management and future directions. – Wildl. Res. 43: 576–589.

Durlak, J. A. 2009. How to select, calculate and interpret effect sizes. – J. Pediatr. Psychol. 34: 917–928.

Ealey, E. H. M. and Main, A. R. 1967. Ecology of the euro, *Macropus robustus* (Gould), in North Western Australia III. Seasonal changes in nutrition. – Wildl. Res. 12: 53–65.

Ellis, B. A. et al. 1977. Seasonal changes in diet preferences of free-ranging red kangaroos, euros and sheep in western New South Wales. – Wildl. Res. 4: 127–144.

Evans, J. and Cushman, S. 2009. Gradient modeling of conifer species using random forests. – Landscape Ecol. 24: 673–683.

Evans, J. S. et al. 2011. Modeling species distribution and change using random forest. – In: Predictive species and habitat modeling in landscape ecology. Springer, pp. 139–159.

Fancourt, B. A. and Nicol, S. C. 2019. Hematologic and serum biochemical reference intervals for wild eastern quolls (*Dasyurus viverrinus*): variation by age, sex and season. – Vet. Clin. Pathol. 48: 114–124.

Finnegan, D. 2015. Package 'referenceIntervals'. – CRAN Repository.

Flatland, B. et al. 2010. ASVCP quality assurance guidelines: control of general analytical factors in veterinary laboratories. – Vet. Clin. Pathol. 39: 264–277.

Fletcher, D. 2007. Managing eastern grey kangaroos *Macropus giganteus* in the Australian Capital Territory: reducing the overabundance – of opinion. – In: Lunney, D. (ed.), Pest or guest: the zoology of overabundance. R. Zool. Soc. of New South Wales, Mosman, NSW, pp. 117–128.

Friedrichs, K. R. et al. 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. – Vet. Clin. Pathol. 41: 441–453.

Gall-Payne, C. et al. 2015. Supersize me: heavy eastern grey kangaroo mothers have more sons. – Behav. Ecol Sociobiol. 69: 795.

Gélin, U. et al. 2015. Experimental manipulation of female reproduction demonstrates its fitness costs in kangaroos. – J. Anim. Ecol. 84: 239–248.

Green-Barber, J. M. et al. 2018. Blood constituents of free-ranging eastern grey kangaroos (*Macropus giganteus*). – Aust. Mammal. 40: 136–145.

Harewood, W. J. et al. 2000. The effects of the menstrual cycle, pregnancy and early lactation on haematology and plasma biochemistry in the baboon (*Papio hamadryas*). – J. Med. Primatol. 29: 415–420.

Harvey, J. W. et al. 1994. Haematological findings in pregnant, postparturient and nursing mares. – Comp. Haematol. Int. 4: 25–29.

Herbert, C. A. 2004. Long-acting contraceptives: a new tool to manage overabundant kangaroo populations in nature reserves and urban areas. – Aust. Mammal. 26: 67–74.

Helle, S. et al. 2008. Female field voles with high testosterone and glucose levels produce male-biased litters. – Anim. Behav. 75: 1031–1039.
Henderson, T. et al. 2018. Population density of eastern grey kangaroos (*Macropus giganteus*) in a periurban matrix at Coffs Harbour, New South Wales. – Aust. Mammal. 40: 312–314.
Hodgkinson, A. and Hambleton, J. 1969. Elevation of serum calcium concentration and changes in other blood parameters after death. – J. Surg. Res. 9: 567–574.
ISIS. 2002. Reference ranges for physiological values in captive wildlife. – International Species Information System.
Jones, M. L. and Allison, R. W. 2007. Evaluation of the ruminant complete blood cell count. – Vet. Clin. North. Am.-Food. Anim. Prac. 23: 377–402.
Kaymaz, A. A. et al. 1999. Serum protein electrophoresis in dogs with intestinal parasites. – Turk. J. Vet. Anim. Sci. 23: 457–460.
King, W. J. et al. 2011. A capture technique for free-ranging eastern grey kangaroos (*Macropus giganteus*) habituated to humans. – Aust. Mammal. 33: 47–51.
Lahti, A. et al. 2002. Objective criteria for partitioning gaussian-distributed reference values into subgroups (Laboratory Management). – Clin. Chem. 48: 338.
Lahti, A. et al. 2004. Partitioning of nongaussian-distributed biochemical reference data into subgroups. – Clin. Chem. 50: 891.
Lakens, D. 2013. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. – Front. Psychol. 4: 863–863.
Lepitzki, D. A. W. and Woolf, A. 1991. Hematology and serum chemistry of cottontail rabbits of southern Illinois. – J. Wildl. Dis. 27: 643–649.
Linton, P. J. and Dorshkind, K. 2004. Age-related changes in lymphocyte development and function. – Nat. Immunol. 5: 133–139.
Maceda-Veiga, A. et al. 2015. Inside the Redbox: applications of haematology in wildlife monitoring and ecosystem health assessment. – Sci. Total Environ. 514: 322–332.
Magona, J. W. and Musisi, G. 2002. Influence of age, grazing system, season and agroclimatic zone on the prevalence and intensity of gastrointestinal strongylosis in Ugandan goats. – Small Ruminant Res. 44: 187–192.
Maher, J. M. et al. 2013. The other half of the story: effect size analysis in quantitative research. – CBE Life Sci. Educ. 12: 345–351.
Mathews, F. et al. 2006. Health surveillance in wildlife reintroductions. – Biol. Conserv. 131: 338–347.
McKenzie, S. et al. 2002. Haematology and serum biochemistry of the tammar wallaby, *Macropus eugenii*. – Comp. Clin. Path. 11: 229–237.
Miller, E. J. et al. 2010. Dominance, body size and internal relatedness influence male reproductive success in eastern grey kangaroos (*Macropus giganteus*). – Reprod. Fertil. Dev. 22: 539–549.
Moss, G. L. and Croft, D. B. 1999. Body condition of the red kangaroo (*Macropus rufus*) in arid Australia: the effect of environmental condition, sex and reproduction. – Austral Ecol. 24: 97–109.
Murphy, M. A. et al. 2010. Quantifying *Bufo boreas* connectivity in Yellowstone National Park with landscape genetics. – Ecology 91: 252–261.
Nakagawa, S. and Cuthill, I. C. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. – Biol. Rev. 82: 591–605.
Pacioni, C. et al. 2013. Hematologic characteristics of the woylie (*Bettongia penicillata ogilbyi*). – J. Wildl. Dis. 49: 816–830.
Peck, S. et al. 2015. Hematologic and serum biochemical reference intervals for wild Tasmanian devils (*Sarcophilus harrisii*). – Vet. Clin. Pathol. 44: 519–529.
Poole, W. E. 1973. A study of breeding in grey kangaroos, *Macropus giganteus* Shaw and *M. fuliginosus* (Desmarest), in central New South Wales. – Aust. J. Zool. 21: 183–212.
Poole, W. E. et al. 1982. Growth of grey kangaroos and the reliability of age determination from body measurements I. The eastern grey kangaroo, *Macropus giganteus*. – Wildl. Res. 9: 9–20.
Portas, T. J. and Snape, M. A. 2018. Factors influencing sub-adult mortality events in eastern grey kangaroos (*Macropus giganteus*) in the ACT. – Environment, Planning and Sustainable Development Directorate, ACT Government, Canberra, <www.environment.act.gov.au>, accessed 18 Apr 2019.
Presidente, P. J. A. 1978. Diseases seen in free-ranging marsupials and those held in captivity. – Proc. Course Vet. Fauna A B 36: 457–471.
Quesnel, L. et al. 2017. Size, season and offspring sex affect milk composition and juvenile survival in wild kangaroos. – J. Zool. 302: 252–262.
Robert, K. A. and Schwanz, L. E. 2013. Monitoring the health status of free-ranging tammar wallabies using hematology, serum biochemistry and parasite loads. – J. Wildl. Manage. 77: 1232–1243.
Sealander, J. A. 1964. The influence of body size, season, sex, age and other factors upon some blood parameters in small mammals. – J. Mammal. 45: 598–616.
Shepherd, N. 1987. Condition and recruitment of kangaroos. – Camb. Stud. Appl. Ecol. Resour. Manage., pp. 135–158.
Sidman, C. L. et al. 1987. Increased expression of major histocompatibility complex antigens on lymphocytes from aged mice. – Proc. Natl Acad. Sci. USA 84: 7624–7628.
Spencer, P. B. S. and Speare, R. 1992. Hematology of wild allied rock-wallabies, *Petrogale assimilis* Ramsay, 1877 (Marsupialia, Macropodidae), in North Queensland. – Aust. J. Zool. 40: 355–364.
Stirrat, S. C. 2003. Body condition and blood chemistry of agile wallabies (*Macropus agilis*) in the wet–dry tropics. – Wildl. Res. 30: 59–67.
Thrall, M. A. et al. 2012. Veterinary hematology and clinical chemistry, 2nd edn. – Wiley-Blackwell.
Vogelnest, L. and Portas, T. 2008. Macropods. – In: Vogelnest, L. and Woods, R. (eds), Medicine of Australian mammals. CSIRO Publishing, pp. 133–225.
Warren, K. S. et al. 2015. Hematologic and serum biochemical reference intervals for the bilby (*Macrotis lagotis*). – J. Wildl. Dis. 51: 889–895.
Wesson Iii, J. A. et al. 1979. Influence of the time of sampling after death on blood measurements of the white-tailed deer. – Can. J. Zool. 57: 777–780.
Wilcox, R. et al. 2011. Gammaherpesvirus infection in a free-ranging eastern grey kangaroo (*Macropus giganteus*). – Aust. Vet. J. 89: 55–57.
Wilson, M. E. and Coulson, G. 2016. Comparative efficacy of levonorgestrel and deslorelin contraceptive implants in free-ranging eastern grey kangaroos (*Macropus giganteus*). – Wildl. Res. 43: 212–219.
Wilson, M. E. et al. 2013. Deslorelin implants in free-ranging female eastern grey kangaroos (*Macropus giganteus*): mechanism of action and contraceptive efficacy. – Wildl. Res. 40: 403–412.
Woolford, L. et al. 2020. Serum biochmistry of free-ranging southern hairy-nosed wombats (*Lasiorhinus latifrons*). – J. Zoo Wildl. Med. 50: 937–946.

Supplementary material (available online as Appendix wlb-00692 at <www.wildlifebiology.org/appendix/wlb-00692>). Appendix 1.