

## Original Contribution

# Temporal Variation in Physiological Biomarkers in Black Flying-Foxes (*Pteropus alecto*), Australia

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**Abstract:** Bats of the genus *Pteropus* (Pteropodidae) are recognised as the natural host of multiple emerging pathogenic viruses of animal and human health significance, including henipaviruses, lyssaviruses and ebolaviruses. Some studies have suggested that physiological and ecological factors may be associated with Hendra virus infection in flying-foxes in Australia; however, it is essential to understand the normal range and seasonal variability of physiological biomarkers before seeking physiological associations with infection status. We aimed to measure a suite of physiological biomarkers in *P. alecto* over time to identify any seasonal fluctuations and to examine possible associations with life-cycle and environmental stressors. We sampled 839 adult *P. alecto* in the Australian state of Queensland over a 12-month period. The adjusted population means of every assessed hematologic and biochemical parameter were within the reported reference range on every sampling occasion. However, within this range, we identified significant temporal variation in these parameters, in urinary parameters and body condition, which primarily reflected the normal annual life cycle. We found no evident effect of remarkable physiological demands or nutritional stress, and no indication of clinical disease driving any parameter values outside the normal species reference range. Our findings identify underlying temporal physiological changes at the population level that inform epidemiological studies and assessment of putative physiological risk factors driving Hendra virus infection in *P. alecto*. More broadly, the findings add to the knowledge of *Pteropus* populations in terms of their relative resistance and resilience to emerging infectious disease.

**Keywords:** Black flying-fox, *Pteropus alecto*, biomarker, haematology, biochemistry, urinalysis

## INTRODUCTION AND PURPOSE

Bats of the genus *Pteropus* (Pteropodidae), colloquially known as flying-foxes, have been identified as a source of a

number of emerging pathogenic viruses of animal and human health significance, including henipaviruses, lyssaviruses, coronaviruses and ebolaviruses (Calisher et al. 2006; Jayme et al. 2015; Smith et al. 2016). Hendra virus, first described in Australia in 1994, causes highly lethal infection in horses and close-contact humans (Murray et al.

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1995). Of the four *Pteropus* species endemic to mainland Australia, there is accumulating evidence that Black (*P. alecto*) and Spectacled (*P. conspicillatus*) flying-foxes are primary reservoir hosts for Hendra virus (Smith et al. 2014; Edson et al. 2015; Goldspink et al. 2015; Field et al. 2015).

Understanding flying-fox-level factors that drive Hendra virus infection dynamics is fundamental to elaborating the disease ecology of the virus and thus effective exposure risk management in horses and humans. Previous studies suggest that physiological and ecological factors, constitute risk factors for infection in flying-foxes (Field 2005; Plowright et al. 2008; Breed et al. 2011), and by extension pose an increased risk of spill-over into other species (Field et al. 2011, 2012; Plowright et al. 2015). However, it is essential to know the normal range of physiological biomarkers and their possible seasonal variability before seeking physiological associations with infection and/or disease.

McMichael et al. (2015) published reference ranges for hematologic and biochemical parameters for *P. alecto*, but there are no published studies on temporal variability of these or other physiological biomarkers in flying-foxes. This study aims to measure physiological biomarkers in *P. alecto* over time to identify any seasonal population and sex-specific fluctuations, and to examine possible associations with life-cycle and environmental stressors.

## METHODS

### Animals, Ethics and Study Sites

Blood and urine samples, and morphometric and reproductive status data were collected from individual adult *P. alecto* during seven bi-monthly catching events between June 2013 and May 2014 at a peri-urban parkland roost at Boonah, in South East Queensland, Australia. The typically seasonal birth pulse in flying-foxes in eastern Australia (late September to early December, peaking in October) precluded adequate temporal representation of immature animals, so only adult animals were included in the analysis. Not all animals voided a urine sample, and the number of blood samples collected was limited by resource constraints.

All applicable institutional and/or national guidelines for the care and use of animals were followed. Fieldwork was conducted under the Queensland Department of Agriculture, Fisheries and Forestry Animal Ethics Com-

mittee Permit SA 2011/12/375, and Department of Environment, Heritage and Protection Scientific Purposes Permits WISP05810609 and WISP14100614.

### Animal Capture, Sample and Data Collection

Bats were captured pre-dawn and anaesthetised as per McMichael et al. (2015). Sex, age class (estimated by morphometric measurements of forearm length (mm) and weight (g), and presence of secondary sexual characteristics) (Epstein et al. 2008) and body condition (assessed by palpation of the pectoral muscle bulk and associated prominence of the sternal carinum; quantified on a 5-point scale where 1 = poor and 5 = good body condition) were recorded. Morphometric index was calculated for each animal (forearm length (mm)/weight (g)). Pregnancy was determined by gentle trans-abdominal palpation, and lactation by expression of milk from the teats. Approximately 3 mL of blood was collected from the propatagial (cephalic) vein and dispensed into a 1.3 mL Lithium Heparin blood tube (Sarstedt 2269201), a 0.5 mL EDTA blood tube (Microtainer 5974) and a direct blood smear prepared and stained using the DiffQuik system (POCD Healthcare). Blood glucose concentration was measured at the time of bleeding using an ACCU-CHEK Performa glucometer (Roche Diagnostics GmbH). Voided urine samples were obtained by trans-abdominal palpation and gentle manual bladder expression. After anaesthesia, each bat was monitored until fully conscious and satisfactory haemostasis at the venepuncture site confirmed prior to placement into a pillowcase for a further recovery period of at least 30 min prior to release at the capture site.

### Hematologic, Biochemical and Urine Analysis

Blood samples were submitted to the Queensland Medical Laboratories (QML), Brisbane, Australia, for haematology. Blood smears from all samples were examined for cell normality. Logistical constraints precluded haematology in June 2013. Plasma samples were also submitted to QML for plasma biochemistry as per McMichael et al. (2015). Urinalysis was conducted at the field site within 4 h of urine collection, using Urispec Plus reagent test strips (Henry Schein 900-3567). Ketone result validation was performed on a subset of 16 samples with negative, low, medium and high ketone readings, using a ketone body assay kit for  $\beta$ -hydroxybutyrate and aceto-acetate (Abnova KA1630). Ur-

ine specific gravity (USG) was measured using a hand-held clinical refractometer. Wet urine mounts were prepared for a subset of six samples that demonstrated high leucocyte readings and stained using Methylene blue (Quick Dip II, Fronine Laboratory Supplies GG023).

### Statistical Analysis

Temporal effects on hematologic, plasma biochemistry and urine parameters were assessed. Where values were reported lower than the detectable limit of the assay, half of the lowest detectable concentration was assigned and used in comparative statistical analyses. Data that proved to be positively skewed with heterogeneous variance were transformed using the natural log (ln). Each variable was subjected to an unbalanced generalised linear model (McCullagh and Nelder 1989), under the normal or log normal distribution as appropriate for continuous variables, and the binomial distribution and logit link for binary variables, using GenStat (2013). Adjusted means and standard errors were estimated for each variable. The seven bi-monthly sampling events were considered as discrete levels of a Month factor in the analysis. Sex of the bat, and the interaction between these factors, was also included in the analyses.

Residual plots for most variables proved to be approximately normal. Mean levels were back-transformed from the ln-scale and reported as population geometric means and significant grouping testing performed.

## RESULTS

A total of 839 adult *P. alecto* were captured and sampled, yielding 406 urine samples, 324 blood samples and 303 plasma samples.

### Reproduction and Body Condition

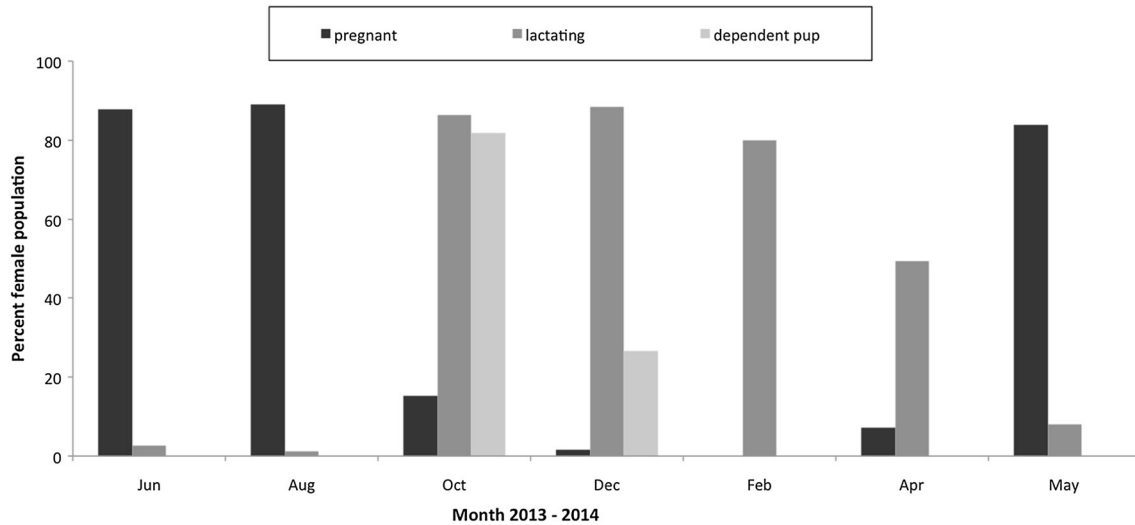
The proportion of palpably pregnant females showed little difference between years from approximately 90% in June 2013 and 85% in May 2014, with little difference in stage of pregnancy at these times (Fig. 1). There was, as expected, a strong seasonal decline in pregnancy to approximately 5% in December, consistent with the typical October birth peak. The proportion of adult females with dependent suckling young showed a strong seasonal increase from 0% in August to approximately 80% in October, followed by a

decline to 0% in February. There was little difference between the proportion of lactating females throughout the post-partum nursing season, approximately 85% in October and 80% in February, declining to approximately 10% in May, reflecting weaning of the increasingly independent young, and coinciding with an increase in pregnancy from 10% in April to 80% in May. Significant statistical correlation was shown for palpably pregnant females in May to August ( $P = 0.001$ ) and lactating females in December ( $P = 0.001$ ).

Average adult female body mass was  $672.7 \pm 87.6$  g, with an average forearm length of  $160.0 \pm 6.0$  mm. Average adult male body mass was  $781.8 \pm 91.1$  g with an average forearm length of  $162.8 \pm 6.1$  mm. Body mass and morphometric index temporal trends were similar, as were the body condition scores, apart from an increased body condition for females in October (Fig. 2). Both male and female body conditions as estimated by morphometric index were dependent on the time of year and demonstrated a significant correlation with month ( $P \leq 0.001$ ) and sex ( $P \leq 0.001$ ). The highest morphometric index in females was estimated in June 2013 and May 2014 and the lowest from August to December. Statistical correlations between morphometric index and pregnancy (April to October) and lactation (October to April) were  $P = 0.075$  and  $P = 0.018$  respectively. In males, the highest morphometric index occurred in February and the lowest in April.

### Haematology

The adjusted bi-monthly mean of each hematologic parameter was within the established reference range for healthy adult *P. alecto* (McMichael et al. 2015). However, with the exception of monocyte and basophil counts, all showed significant temporal variation between adjusted bi-monthly means (Table 1; Fig. 3). Significant differences were observed between the sexes for mean corpuscular haemoglobin (MCH) and platelet count. Significantly higher haemoglobin levels were observed for females in May, while males had significantly lower levels in October and April. Red cell count (RCC) followed a similar trend where females were observed to have significantly higher counts in April and May, and males a significant decrease in October. Neutrophil counts in females increased significantly in October, while male neutrophil counts demonstrated only minor temporal changes across the study. Female lymphocyte counts increased significantly in May, while male lymphocyte counts were elevated in December and May.



**Figure 1.** Reproductive status of adult female *Pteropus alecto* (2013: June  $n = 86$ , August  $n = 55$ , October  $n = 67$ , December  $n = 70$ . 2014: February  $n = 11$ , April  $n = 70$ , May  $n = 36$ ).

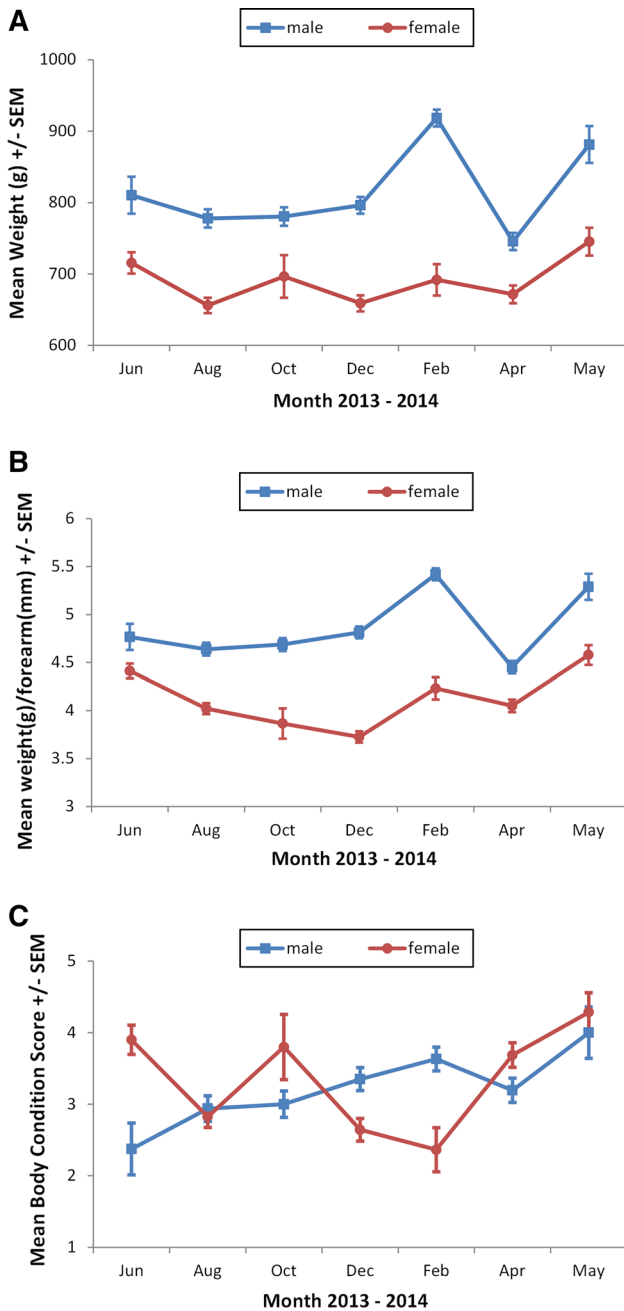
### Plasma Biochemistry

Similarly, the adjusted bi-monthly mean of each biochemical parameter was within the established reference range for healthy adult *P. alecto* (McMichael et al. 2015). However, again there was significant temporal variability between adjusted bi-monthly means (Table 1; Fig. 4). Significant differences were observed between the sexes for phosphorous, creatinine, alanine transaminase (ALT), alkaline phosphatase (ALP), albumin, glucose, cholesterol and triglycerides. While males demonstrated low-magnitude temporal variation in triglycerides and cholesterol, females, apart from a significant increase in triglycerides in May, demonstrated significantly higher triglyceride levels in February and June, and higher cholesterol levels in February, June and August. Glucose was significantly higher in females in February, and in males in December. Creatinine in females was significantly elevated from April to June, but in males in April and May only. Elevated levels of gamma-glutamyl transferase (GGT) in females in April and May were not statistically significant, but those in males in the same months were. ALP in females showed a significant decrease in February, with increased levels in April and May; male ALP levels demonstrated significant increases in December, April and May. The only significant fluctuations in electrolyte levels were significant increases in female sodium and chloride levels in February, and significant decreases in female calcium levels in December.

### Urinalysis

All examined parameters, with the exception of urinary nitrite, showed significant temporal variations between adjusted bi-monthly means (Table 1; Fig. 5). Significant differences were observed between the sexes for urine specific gravity (USG) and urinary ketones, pH, protein, blood, leucocytes and nitrite. Females demonstrated significantly lower USG in December; in males, USG was significantly higher in February. Males showed significant elevations in urinary ketones from December to May; female levels were significantly lower than males and revealed no significant temporal variation. Females demonstrated significantly higher urinary pH from August to April, while in males, urinary pH was consistently lower with no significant temporal changes. Males had significantly higher urinary leucocyte levels in April and May, while females had consistently low levels with no significant temporal change.

Approximately 29, 36, 40 and 95% of urine samples returned positive results for blood, glucose, protein and ketones respectively. The presence of urobilinogen, bilirubin and nitrites in urine was rare, with approximately 1, 3 and 2%, respectively, of urine samples returning positive results. Ketone dipstick results demonstrated correlation with concentration of aceto-acetate ( $r^2 = 0.72$ ) and no correlation with concentration of beta-hydroxybutyrate ( $r^2 = 0.02$ ). Microscopic examination of urine samples that returned positive results for leucocytes found less than five



**Figure 2.** Bodyweight (a), morphometric index (b) and body condition score (c) of adult *Pteropus alecto*

leucocytes per high-powered field (HPF). All urine sediment preparations examined revealed occasional anucleate squamous epithelial cells (less than 5/HPF).

## DISCUSSION

This study sought to identify and describe temporal variations in key physiological biomarkers in *P. alecto*, and determine whether such changes could be explained by

natural life-cycle events, or whether they indicated environmental stressors such as food availability or seasonal climatic conditions. We found significant temporal variation in numerous physiological biomarkers between sampling events and within sampling events, evidently associated with gender, reproductive status or body condition.

Body condition is an important indicator of an animal's fitness, usually referable to magnitude of energy stores relative to the structural size of the animal (Green 2001). The use of morphometric index as a sole indicator of body condition has been the subject of contention (Green 2001), and thus, we additionally recorded body mass and body condition score. We found that the differing measures used to establish body condition demonstrated similar temporal trends. Fitness-maximising strategies of males and females typically differ, particularly with respect to timing of maximal reproductive effort, resulting in sex-specific seasonal cycles in body mass, body condition and fat deposition (Lindstedt and Boyce 1985; Welbergen 2011). In this study, we demonstrated that female body condition declined during the lactation period and increased as the young was weaned. This likely reflects the high metabolic load that lactation imposes on nursing dams, and is consistent with the general observation that lactation is the most energetically demanding period across all mammalian species (Millar 1977; Thometz et al. 2014). In contrast, males accumulated body reserves prior to the breeding season but subsequently lost body mass during the peak mating season (March and April), likely due to territory defence, courtship and lost foraging opportunities (Welbergen 2011). As body condition followed distinctly different seasonal patterns for each sex, food availability or environmental conditions are unlikely to be the primary driver of body condition in this species at these times.

The temporal trends in red cell count and haemoglobin follow similar temporal trends as body condition for each sex, with a trend towards lower values in October for females during birthing and early lactation followed by a steady increase during weaning and pregnancy. Males demonstrated an increasing trend during the lead up to mating between December and February, followed by a low point during peak mating. This finding is consistent with other wildlife studies which have demonstrated that erythrocyte parameters are closely correlated with body condition (Algar et al. 1988; Ruykys and McCarthy 2012).

Sex-related differences in observed temporal trends for lymphocyte and neutrophil counts might be related to

**Table 1.** General Linear Model analysis of adult *Pteropus alecto* Hematologic, Biochemical and Urinalysis Parameter Population Means with Sex and Time (Significance Level  $P < 0.05$ )

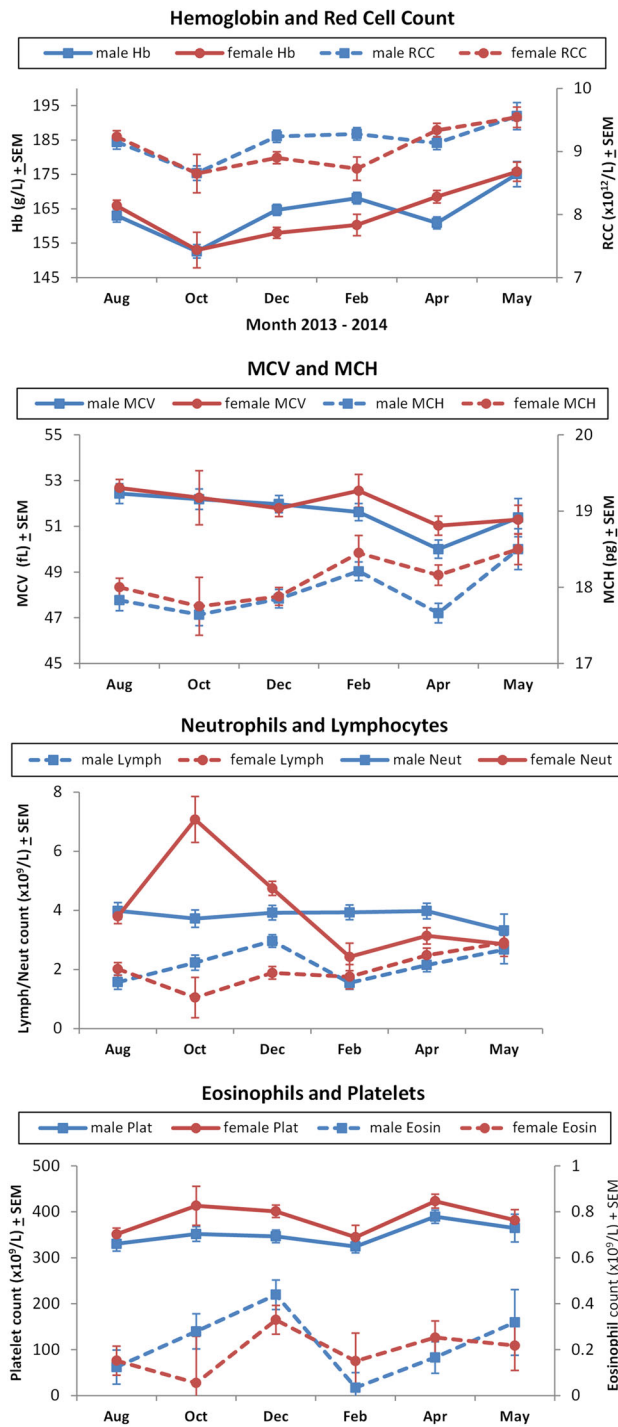
Parameter	Month	Sex	Month.Sex
<b>Haematology</b>			
Haemoglobin	< <b>0.001</b>	0.778	< <b>0.001</b>
Red cell count	< <b>0.001</b>	0.179	<b>0.019</b>
Mean corpuscular volume	< <b>0.001</b>	0.204	0.665
Mean corpuscular haemoglobin	< <b>0.001</b>	<b>0.026</b>	0.537
Platelets	< <b>0.001</b>	< <b>0.001</b>	0.799
Neutrophil count	<b>0.002</b>	0.763	< <b>0.001</b>
Lymphocyte count	< <b>0.001</b>	0.380	<b>0.004</b>
Monocyte count	0.476	0.359	0.234
Eosinophil count	< <b>0.001</b>	0.858	0.496
Basophil count	0.090	0.515	0.815
<b>Biochemistry</b>			
Sodium	<b>0.032</b>	0.609	<b>0.006</b>
Chloride	< <b>0.001</b>	0.542	<b>0.003</b>
Potassium	< <b>0.001</b>	0.286	0.555
Phosphorous	< <b>0.001</b>	<b>0.010</b>	0.055
Calcium	< <b>0.001</b>	0.146	<b>0.048</b>
Bicarbonate	< <b>0.001</b>	0.352	0.190
Anion gap	< <b>0.001</b>	0.480	0.051
Urea	< <b>0.001</b>	0.997	0.065
Creatinine	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>
Bilirubin	< <b>0.001</b>	0.328	0.130
Aspartate aminotransferase	< <b>0.001</b>	0.155	0.190
Alanine transaminase	< <b>0.001</b>	<b>0.022</b>	0.620
Gamma-glutamyl transferase	< <b>0.001</b>	0.203	<b>0.015</b>
Alkaline phosphatase	< <b>0.001</b>	< <b>0.001</b>	<b>0.021</b>
Creatinine kinase	< <b>0.001</b>	0.167	0.400
Albumin	< <b>0.001</b>	<b>0.017</b>	0.140
Globulin	< <b>0.001</b>	0.473	0.446
Glucose	<b>0.003</b>	< <b>0.001</b>	<b>0.039</b>
Cholesterol	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>
Triglycerides	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>
<b>Urinalysis</b>			
USG	< <b>0.001</b>	< <b>0.001</b>	<b>0.022</b>
Ketones	< <b>0.001</b>	< <b>0.001</b>	<b>0.002</b>
pH	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>
Protein	< <b>0.001</b>	<b>0.002</b>	0.098
Glucose	<b>0.005</b>	0.547	0.272
Blood	<b>0.014</b>	< <b>0.001</b>	0.062
Leucocytes	< <b>0.001</b>	< <b>0.001</b>	<b>0.028</b>
Nitrite	0.708	<b>0.002</b>	0.463

Bold values are statistically significant

respective male and female reproductive cycles. Neutrophil counts were highest and lymphocyte counts lowest for females during the birthing season and early lactation con-

sistent with a peri-parturient cortisol spike observed in well-studied domestic species (Guidry et al. 1976). Examination of blood smears from female animals in October





**Figure 3.** Hematologic parameters of adult *Pteropus alecto* (sex-specific bi-monthly population mean ( $\pm$ SEM)) (logistical constraints precluded haematology in June 2013).

confirmed that all neutrophil populations were mature, suggesting that the increased neutrophil counts during this time were most likely due to a redistribution effect as a result of cortisol or sympathetic nervous system activation, and not part of an active inflammatory response.

Although statistically significant, the magnitude of temporal changes in glucose, albumin, triglyceride and cholesterol levels were still relatively minor and within the established normal reference ranges, most likely reflecting cyclic physiological changes and seasonal diet content. As *P. alecto* is predominantly frugivorous, feeding on both fruit and blossom (Jackson 2003; Churchill 2008), the higher blood glucose during the summer months plausibly reflects fruit being a major dietary component at this time. The increasing trend of plasma albumin in the winter months might be due to increased availability of blossom, yielding a higher protein food source (Roulston and Cane 2000).

Interpretation of the temporal changes in plasma triglycerides and cholesterol is initially challenging since *P. alecto* has a low fat diet and thus very low levels of circulating fatty acids (Riedesel 1977). The changes most plausibly indicate mobilisation and depletion of lipid energy stores during demanding stages of the life cycle. This is particularly evident in the decreasing triglyceride and cholesterol levels in pregnant females transitioning to lactation, followed by an increase in triglycerides around the time of weaning, consistent with findings in other domestic and wild mammal species (Srivastava and Krishna 2008; Kessler et al. 2014). Males tended to have slightly decreased levels of triglycerides in April, possibly attributed to altered trafficking as a result of the high metabolic demand of peak mating, but otherwise demonstrated little change in triglyceride and cholesterol levels.

The elevation of ALT, AST, GGT and ALP enzyme activity in females in October might plausibly be associated with the physiological demands of birthing and early lactation, which could place increased metabolic load upon the liver and other tissues, while in males, the December elevation may be associated with the switch in physiological demand to an anabolic state associated with the build-up in body reserves for the mating season, the physiological demands of movement to a breeding colony, establishing territory and breeding harems and potentially differing foraging behaviour during this time.

Creatinine and urea levels were also generally higher across the autumn and winter months from April to August, and GGT and ALP activity higher in April and May, possibly due to greater physiological demands of thermoregulation, lower levels of hydration, increased hepatocellular enzyme activity associated with increased protein intake or the effects of pregnancy on females and the physiological demands during peak mating in Autumn for males. However, the elevations were not of a magnitude

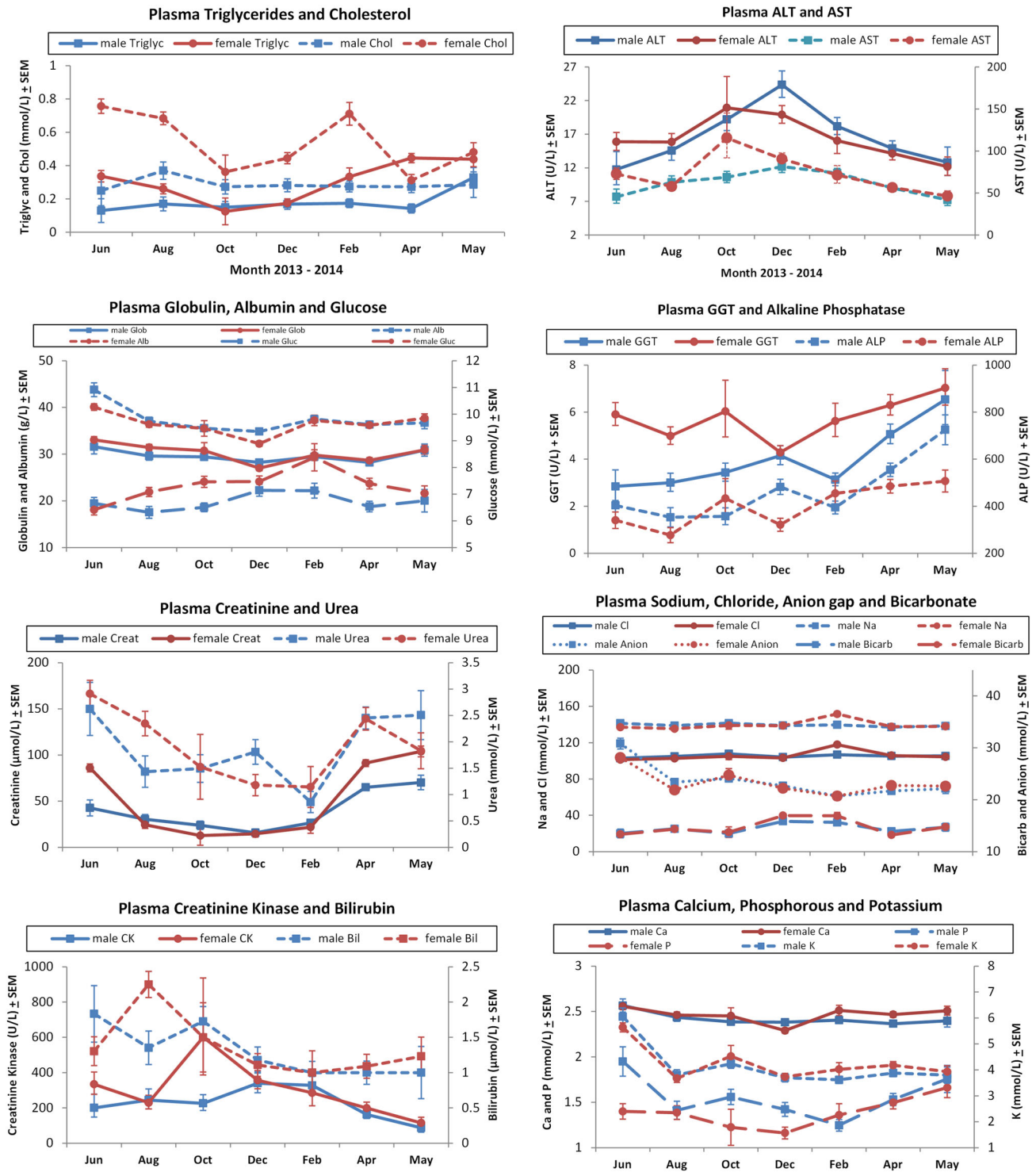


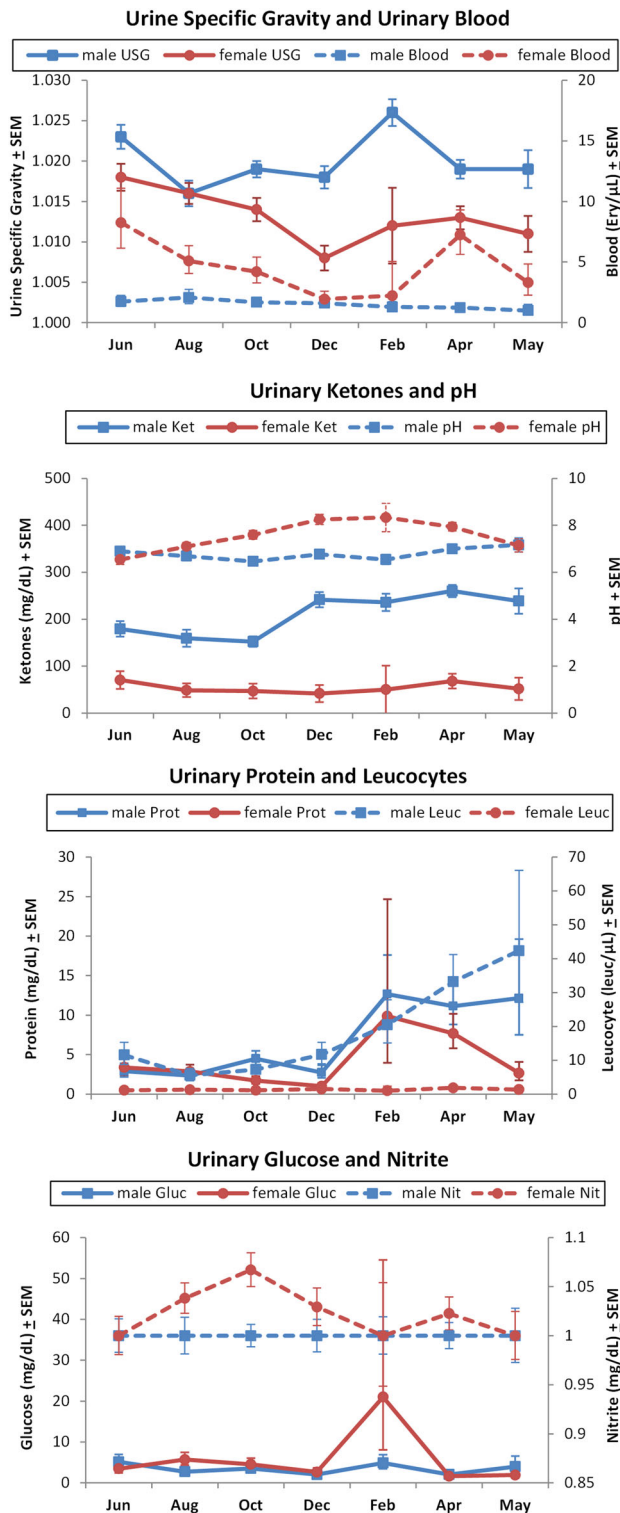
Figure 4. Plasma biochemistry parameters of adult *Pteropus alecto* (sex-specific bi-monthly population mean (±SEM)).

consistent with nutritional stress or obvious tissue insult and are most likely to be reflective of life-cycle stages. Females also exhibited temporal trends in calcium and phosphorous levels consistent with lactation and weaning, and both sexes exhibited mild elevations in bicarbonate

during summer which might be associated slight fluctuations in acid–base homeostasis and osmoregulation.

The presence of urinary ketones is usually an abnormal clinical finding in mammals. We suggest the high levels of ketones in male urine are likely linked to glandular





**Figure 5.** Urinary parameters of adult *Pteropus alecto* (sex-specific bi-monthly population mean ( $\pm$ SEM)).

excretions associated with the build-up to peak mating, and have previously been reported in the urogenital secretions of the paraphyletic *P. conspicillatus* (Wagner 2008). If ketogenesis was the source of urinary ketones, evidence of

mobilisation of triglycerides and declining body condition could be expected. However, there were significant increases in male body condition, more indicative of an anabolic metabolic state prior to mating, when urinary ketone levels were highest. There was also no evidence that the presence of urinary ketones in male *P. alecto* was associated with a clinically significant metabolic acidosis.

Males showed limited presence of blood in urine and females showed significantly higher levels of blood in the urine during peak mating and pregnancy, plausibly associated with copulation and early pregnancy. The higher urine protein levels in males and females in February and April plausibly reflect peak mating period and the presence of spermatozoa in urine. Alternatively, mild elevations of protein levels in both sexes could theoretically be attributed to mild transient renal functional abnormalities following a severe heat stress event in South East Queensland in January 2014 (Welbergen 2014); however, there was no concomitant hypoalbuminemia consistent with a clinically significant protein-losing glomerulonephritis. The increase in urinary glucose levels in females in February 2014, could also theoretically be explained by the same aforementioned heat stress event, although the elevations were not found to be statistically significant, nor were elevations observed in males.

The temporal change in female urine pH may be indicative of differing availability or preference for a high carbohydrate fruit diet during the summer months while lactating. A more acidic urinary pH is usually indicative of a higher dietary acid load (typically higher protein content), while more alkaline pH is indicative of a higher carbohydrate diet (MacIntosh et al. 2012). However, the differing urine pH profiles between males and females is most plausibly due to the differing physiological status between the sexes, in particular lactation in females, rather than differing diets, as there is no evidence supporting differing foraging behaviours between the sexes of *P. alecto* (Palmer and Woinarski 1999; Markus and Hall 2004).

The calculated reference range of USGs of individual *P. alecto* of 1.001 to 1.037 demonstrates lower values when compared to normal reference ranges of rats, dogs and cats (Johnson-Delaney 1996; Merck 2015), and just over 24% of all measured USGs were in the isosthenuric range of dogs and cats (1.008–1.012). Two percent of values were found to be equal to or above 1.040, suggesting that a small minority of animals returning from nightly foraging may be under-hydrated. Studier and Wilson (1983) found that the urine concentrating ability of nectarivorous and frugivo-

rous bats fell below that of insectivorous bats, suggesting that frugivorous bats are poorly adapted to produce concentrated urine, while Bakken et al. (2008), in studies of a nectarivorous bat, suggest that water and electrolyte balance are maintained by eliminating excess ingested water by reducing renal water reabsorption, and limitation of urinary water loss during fasting by reducing glomerular filtration rate.

We observed an elevated USG in males during February, which may be consistent with a reduced hydration status attributable to territorial defence and harem establishment prior to the peak mating season in April. In females, USG was uniformly low during lactation but high during pregnancy, when it is accepted that blood volumes in mammals commonly increase and conservation of fluids occur. The low USG values during lactation may alternatively reflect a high intake of fluid to support the physiologically demanding life-cycle event of lactation. The significant temporal differences between USG in males and females suggest that hydration status was more reflective of physiological and behavioural factors rather than environmental availability of water, and does not suggest any renal pathology.

While significant temporal changes to several hematologic and biochemical parameters were observed in this study, all observations remained within established reference ranges. The temporal changes observed are plausibly explained by life-cycle stage, and fundamentally do not reflect external stressors. However, we acknowledge that our study period was of limited duration, and that in other years, additional variation could be imposed by negative external factors.

## CONCLUSION

Our findings indicate that the temporal variation in body condition, hematologic, biochemical and urinalysis parameters are consistent with the physiological and energetic demands of life cycle and not fundamentally driven by environmental stressors such as resource availability or normal seasonal climactic conditions. We found no evident effect of remarkable physiological or nutritional stress, and no indication of clinical disease driving any parameter values outside the normal species reference ranges. As both ecological processes and physiological status can influence patterns of infection and/or disease risk, our findings identify underlying temporal physiological

variation at the population level that usefully informs epidemiological studies. In the Australian context, the findings will be valuable in assessing putative physiological risk factors driving Hendra virus infection in *P. alecto*. More broadly, this study adds to the knowledge of *Pteropus* populations in terms of their relative resistance and resilience to emerging infectious disease.

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