

## Haematologic reference ranges of two remnant populations of the red-crowned parakeet (*Cyanoramphus novaezelandiae*) in New Zealand

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**Abstract** We present counts of white blood cells of wild and clinically normal red-crowned parakeets (*Cyanoramphus novaezelandiae*) from 2 island populations in New Zealand. Total white blood cell counts on slides prepared in the field and counts of relative proportion of basophils, eosinophils, heterophils, lymphocytes and monocytes were determined for 33 individuals caught on Little Barrier Island and 48 individuals caught on Raoul Island. Mean haematological parameters were: total white blood cells  $6.85 \times 10^9/L$ , lymphocytes  $5.0 \times 10^9/L$  (74.0%), monocytes  $3.5 \times 10^9/L$  (5.7%), eosinophils  $4.6 \times 10^9/L$  (6.4%), basophils  $1.9 \times 10^9/L$  (3.1%), and heterophils  $9.9 \times 10^9/L$  (14.7%). Raoul Island parakeets had significantly higher counts of white blood cells, lymphocytes, and heterophils than Little Barrier Island parakeets, possibly reflecting latitudinal differences. Males showed significantly higher counts of white blood cells and lymphocytes than females. White blood cell counts on slides prepared in the field represent an inexpensive and straightforward technique to determine variation in the levels of each cell type and to assess the physiological state of healthy and diseased individuals. This information is useful for veterinary clinicians, wildlife managers, and conservation biologists who increasingly require methods for health assessment, disease diagnosis, and screening for pathogenic microorganisms on species of conservation concern.

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**Keywords** haematology; Little Barrier Island; Raoul Island; parakeet; white blood cells

### INTRODUCTION

The conservation of many rare, endemic, and endangered species in New Zealand has 2 major components: (1) control and/or eradication of introduced predators, and (2) translocation to managed habitats (Armstrong & McLean 1995; O'Donnell 1996; Clout & Russell 2006). Increasingly, the screening for pathogenic microorganisms

and other means of health assessment are carried out on the individuals translocated from the wild (Gartrell *et al.* 2006; Parker *et al.* 2006) or from captive-breeding programmes (Jakob-Hoff 2001). In particular, haematologic reference values can assist in the diagnosis of many diseases (Campbell 1994) and in the identification of deviations from normal health status of individuals (Moreno *et al.* 1998).

Recently, the usefulness of white blood cell counts to infer immune capacity of species of conservation interest has been demonstrated in

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**Table 1.** Hematologic reference values for free-living red-crowned parakeets on Little Barrier Island and Raoul Island.

Hematologic count	<i>n</i>	mean $\pm$ SD	Range
White blood cells ( $\times 10^9/L$ )	81	6.85 $\pm$ 3.64	1.60 – 16.40
Lymphocytes ( $\times 10^9/L$ )	79	5.0 $\pm$ 2.84	1.10 – 12.40
Lymphocyte (%)	80	74 $\pm$ 13.97	27 – 98
Monocytes ( $\times 10^9/L$ )	46	0.35 $\pm$ 0.35	0 – 1.80
Monocytes (%)	47	5.70 $\pm$ 4.8	1 – 20
Eosinophils ( $\times 10^9/L$ )	74	0.46 $\pm$ 0.59	0 – 3
Eosinophils (%)	74	6.39 $\pm$ 5.77	1 – 26
Basophils ( $\times 10^9/L$ )	48	0.19 $\pm$ 0.21	0 – 1
Basophils (%)	48	3.08 $\pm$ 2.90	1 – 14
Heterophils ( $\times 10^9/L$ )	79	0.99 $\pm$ 0.94	0 – 5.4
Heterophils (%)	79	14.66 $\pm$ 10.11	1 – 58

Forbes' parakeets (*Cyanoramphus forbesi*) (Tompkins *et al.* 2006) and New Zealand robins (*Petroica longipes*) (Hale & Briskie 2006). While a number of studies have surveyed for a range of microorganisms of interest using blood (Brangenberg *et al.* 2003; Ewen *et al.* 2007; Ortiz-Catedral *et al.* 2009a; Ortiz-Catedral *et al.* 2009b), little information is available on baseline haematological values that could be used as a reference for native New Zealand bird species (Low *et al.* 2006).

The red-crowned parakeet (*Cyanoramphus novaezelandiae*) is a vulnerable species (www.iucn.org) endemic to New Zealand (Boon *et al.* 2001) and occurs from the Kermadec Archipelago to New Zealand's sub-Antarctic islands (Higgins 1999). The species is commonly kept in zoos and private avicultural collections under permit by the New Zealand Department of Conservation. The species has also been translocated to several locations free of introduced mammalian predators throughout the country (McHalick 1999).

We carried out a study to provide baseline reference values for haematologic parameters, specifically white blood cell counts of free-ranging healthy individuals at 2 remnant native populations for the species: Little Barrier Island and Raoul Island. Our aim was to establish standards to assist with medical diagnosis of sick birds held at collections and for reference during future planned translocations of red-crowned parakeets.

## METHODS

We obtained blood samples from yearlings and adult red-crowned parakeets identified in the field based on plumage features (see Higgins 1999) captured using mist nets on Little Barrier Island (LBI) (5 to 17 May 2008) and Raoul Island (RI) (28 March to 16 April 2008). These locations are the focus of our current research on the conservation

and management of the species (Ortiz-Catedral & Brunton 2009; Ortiz-Catedral *et al.* 2009c), and thus mist-netted birds were available for the collection of blood samples and the preparation of blood smears in the field. To prevent re-sampling of the same individual, every captured bird was given a uniquely numbered metal band following guidelines by the New Zealand Department of Conservation.

Blood was obtained by the puncture of the brachial vein. One drop of blood was collected with a non-heparinised capillary tube and transferred onto a microscope slide and smeared to a thin layer following the "push-slide" method (Walberg 2001). We chose non-heparinised capillary tubes to ensure better staining of the slides in the laboratory (Walberg 2001). Slides were air-dried and fixed in 100% methanol (Bennett 1970). Slides were transported to Massey University, Albany Campus, and stained with May Grunwald-Giemsa followed with a phosphate buffer/rinse (Robertson & Maxwell 1990) (Technecult Laboratories Ltd., Napier, New Zealand), and a cover slip added after staining.

For white blood cell counts, we followed the Leukocyte Estimate from Blood Smears Method (LEFS) (Fudge, 2000), and described in Parker *et al.* (2006). Briefly, 10 monolayer fields (*i.e.*, areas where cells are spread out evenly) per slide at 40x magnification were analysed for as long as it took to count 100 leukocytes per slide. Slides unsuitable for examination (*i.e.*, hemolysed or poorly stained) were excluded. Inspection of blood cells for identification of haemoparasites was also conducted alongside. The estimated number of cell types  $N$  ( $\times 10^9/L$ ) was determined using the formula (Fudge 2000):

$$N = (\text{leukocyte count/number of fields}) \times 2$$

Leukocyte counts are the raw values, and the number of fields is at high power. Blood cell counts were completed at Gribbles Veterinary

**Table 2.** Hematologic counts for red-crowned parakeets on Little Barrier Island (LBI) and Raoul Island (RI). Statistical tests performed on transformed data. See methods for details.

Hematologic count	Site	<i>n</i>	Mean $\pm$ <i>SD</i>	Mean rank	Test statistic	<i>P</i>
Basophils (%)	LBI	23	3.65 $\pm$ 3.38	27.22	U-test 1.29	0.12
	RI	25	2.56 $\pm$ 2.33	22		
Basophils ( $\times 10^9/L$ )	LBI	17	0.20 $\pm$ 0.25	23.48	U-test 1.06	0.29
	RI	24	0.18 $\pm$ 0.17	25.44		
Eosinophils (%)	LBI	29	6.03 $\pm$ 5.32	37.12	U-test 0.12	0.90
	RI	45	6.62 $\pm$ 6.09	37.74		
Eosinophils ( $\times 10^9/L$ )	LBI	28	0.29 $\pm$ 0.23	34.10	U-test 0.86	0.39
	RI	45	0.56 $\pm$ 0.72	39.70		
Heterophils (%)	LBI	32	14.25 $\pm$ 11.53	37.78	U-test 0.71	0.48
	RI	47	14.94 $\pm$ 9.14	41.51		
Lymphocytes (%)	LBI	33	73.24 $\pm$ 14.93	-	<i>t</i> -test 0.37	0.71
	RI	47	74.55 $\pm$ 13.40	-		
Monocytes (%)	LBI	24	6.96 $\pm$ 5.70	26.29	U-test 1.17	0.24
	RI	23	4.39 $\pm$ 3.28	21.61		
Monocytes ( $\times 10^9/L$ )	LBI	21	0.32 $\pm$ 0.29	23.12	U-test 0.58	0.56
	RI	22	0.37 $\pm$ 0.41	23.90		

Pathology (Auckland, New Zealand). We used this methodology because it can be applied to slides prepared in the field, and for samples collected in different sampling occasions. Other blood cell count methods require larger amounts of fresh material, and thus are inapplicable in a field situation. The following haematologic parameters were determined: total white blood cell counts, and the percentages of basophils, eosinophils, heterophils, lymphocytes, and monocytes. For a number of slides it was not possible to count all haematologic parameters due to the quality of the preparation and colouration. As a result, the sample size varied between different hematologic counts.

For all blood cell counts, we only used slides from individuals without external signs of sickness or poor body condition (*i.e.*, underweight, extreme feather loss, *etc.*). We also excluded individuals yielding positive results for *Plasmodium* (Ortiz *et al.*, 2011) and Psittacine Beak and Feather Virus (BFDV) screened as part of a separate study documenting the occurrence of these and other pathogens in red-crowned parakeets (Ortiz-Catedral *et al.* 2009b). Further, 69 out of the total 81 samples included in this study (85%), did not test positive for *Campylobacter*, *Salmonella* or *Yersinia* in a separate pathogen screening (Ortiz-Catedral *et al.* 2009a). In addition to blood, 2 contour feathers from the ventral region were collected for molecular determination of sex following the methodology described by Griffiths *et*

*al.* (1998). Molecular sexing was done at the Equine, Parentage and Animal Genetics Centre, Massey University.

### Statistical analysis

We tested for normality and equality of variances in our dataset using the Shapiro-Wilk normality test in SAS version 9.1<sup>®</sup> and equality of variances F test in StatView version 5.0.1<sup>®</sup>. When data deviated significantly from normality ( $P=0.05$ ), we applied an arcsine-square root transformation for proportional data and log transformation for count data. We applied unpaired *t*-tests and Mann-Whitney U tests (on non-transformed data) for comparisons between localities and gender categories. Results are presented as means  $\pm$  SD of untransformed data. Also, for non-parametric comparisons, sum of ranks are included for reference.

### RESULTS

The overall haematologic ranges obtained from free-living, and clinically normal red-fronted parakeets are summarised in Table 1. Haematologic comparisons between LBI and RI parakeets revealed significant differences in white blood cells ( $\times 10^9/L$ ; LBI 5.30 (*SD* 2.82); RI 7.91 (*SD* 3.79);  $t=3.70$ ,  $P<0.01$ ), lymphocytes counts ( $\times 10^9/L$ ; LBI 3.98 (*SD* 2.50); RI 5.73 (*SD* 2.87);  $t=3.27$ ,  $P<0.01$ ) and heterophils counts ( $\times 10^9/L$ ; LBI 0.76 (*SD* 0.63); RI 1.20 (*SD* 1.07);

**Table 3.** Hematologic counts for male (M) and female (F) red-crowned parakeets on Little Barrier Island and Raoul Island. Statistical tests performed on transformed data. See methods for details.

Haematologic count	Sex	<i>n</i>	Mean $\pm$ SD	Mean rank	Test statistic	<i>P</i>
Basophils (%)	M	18	3.22 $\pm$ 3	24.75	<i>U</i> test 0.16	0.87
	F	30	3 $\pm$ 2.89	24.08		
Basophils ( $\times 10^9/L$ )	M	18	0.24 $\pm$ 0.23	21.85	<i>U</i> test 1.72	0.08
	F	30	0.16 $\pm$ 0.19	28.92		
Eosinophils (%)	M	29	5.93 $\pm$ 5.67	38.60	<i>U</i> test 0.55	0.58
	F	45	6.69 $\pm$ 5.88	35.79		
Eosinophils ( $\times 10^9/L$ )	M	29	0.56 $\pm$ 0.73	36.19	<i>U</i> test 0.40	0.69
	F	45	0.39 $\pm$ 0.48	38.22		
Heterophils (%)	M	32	15.22 $\pm$ 10.48	39.62	<i>U</i> test 0.18	0.86
	F	47	14.28 $\pm$ 9.95	40.56		
Heterophils ( $\times 10^9/L$ )	M	32	1.17 $\pm$ 1.06	-	<i>t</i> -test 1.36	0.18
	F	47	0.88 $\pm$ 0.84	-		
Lymphocytes (%)	M	48	75.31 $\pm$ 11.78	-	<i>t</i> -test 0.68	0.50
	F	32	73.15 $\pm$ 15.32	-		
Monocytes (%)	M	16	6.32 $\pm$ 5.38	24.79	<i>U</i> test 0.55	0.58
	F	31	4.50 $\pm$ 3.25	22.47		
Monocytes ( $\times 10^9/L$ )	M	16	0.40 $\pm$ 0.20	22.09	<i>U</i> test 0.01	0.99
	F	30	0.32 $\pm$ 0.29	21.97		

$t = 2.74$ ,  $P < 0.01$ ). All other hematologic parameters (counts and percentages of other blood cell types) were statistically similar on both populations (Table 2). Between sexes, males showed higher counts of white blood cells ( $\times 10^9/L$ ; males 8 (SD 3.77)  $n = 32$ ; females 6.09 (SD 3.38)  $n = 49$ ;  $t = 2.53$   $P = 0.01$ ) and lymphocytes ( $\times 10^9/L$ ; males 5.98 (SD 2.86)  $n = 32$ ; females 4.33 (SD 2.66)  $n = 47$ ;  $t = 2.89$   $P < 0.01$ ) than females. The remaining of hematologic comparisons did not differ significantly between sexes (Table 3).

## DISCUSSION

In this study we established the first haematologic reference values for healthy and wild red-crowned parakeets from native populations. Due to the popularity of parrots as companion animals there is information available on the haematology of numerous captive psittacines in the veterinary literature (Polo *et al.* 1998), but only limited information from field studies. Differences between captive and wild psittacines in haematologic parameters may possibly be associated with sedentary lifestyle and the diet of captive animals (Deem *et al.* 2005). Such patterns highlight the limited clinical utility of haematologic reference values obtained from captive birds when health

assessment and diagnosis is needed for wild individuals. Thus, our contribution is a valuable addition to the growing research on health and disease management of species of conservation concern in New Zealand.

In general, the parameters we observed are within reported ranges in the literature for parrots. In our study, the most common leukocytes were lymphocytes which is consistent among psittacines (Deem *et al.* 2005; Foldenauer *et al.* 2007), although for some species heterophils have been reported as more abundant, making up to 61% of leukocytes in orange-bellied parrots (*Neophema chrysogaster*) (Melrose *et al.* 1995). The pattern of greater abundance of heterophils than other leukocytes has also been documented for budgerigars (*Melopsittacus undulatus*), African grey parrots (*Psittacus eritachus*), cockatiels (*Nymphicus hollandicus*) (Rosskopf *et al.* 1982a; Rosskopf *et al.* 1982b; Rosskopf *et al.* 1982c), and the kakapo (*Strigops habroptilus*) (Low *et al.* 2006), although with the exception of the last species, all the other studies were based on captive birds.

The relative abundance of leukocytes can vary between age groups (Dujowich *et al.* 2005) and environmental conditions (Campbell 1994). Thus, our reference values are indicative of the

proportions of different blood cells of adult red-crowned parakeets towards the end of the breeding season which typically extends from October to March (Greene 2003; Ortiz-Catedral & Brunton 2009). Ideally, a more comprehensive set of reference values should include nestlings and juveniles, as well as adults during both the breeding and the non-breeding seasons. The ease of slide preparation in a field situation means that a more detailed picture of the haematology of wild red-fronted parakeets is achievable and should be the subject of future conservation and basic biological research efforts.

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