

## Blood parameters of migratory shorebirds at Peninsula Valdés, Patagonia

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We present blood parameters related to body condition and immune function of three shorebird species, Red Knot *Calidris canutus rufa*, White-rumped Sandpiper *Calidris fuscicollis* and Two-banded Plover *Charadrius falklandicus*, at a feeding site in Peninsula Valdés, Patagonia, Argentina, during their non-breeding season. We determined concentrations of glucose, cholesterol, and triglycerides, the leukocyte profile and the heterophil/lymphocyte ratio. These data constitute the first report of these blood parameters in shorebirds at this location. These data provide baseline information for ecological studies on these species in this area. The data could also be used in larger meta-analyses comparing blood parameters across geographical areas, different species and over time.

### Keywords

Argentina  
biochemistry  
hematology  
*Calidris canutus*  
*Calidris fuscicollis*  
*Charadrius falklandicus*

## INTRODUCTION

Physiological data is increasingly used to monitor wildlife populations in the face of rapidly changing environments (Cooke *et al.* 2013, Madliger & Love 2015). The use of physiological parameters has become important because they can alert us to changes in the status of individuals allowing for early detection of problems and potentially the chance to mitigate them (Carey 2005). Therefore, these data can be used for conservation decision-making. Given the fragility of the environments and the decline of shorebird populations throughout the Western Hemisphere (Morrison *et al.* 2001), information on physiological parameters could provide baseline information for ecological studies and could also be used in larger analyses comparing blood parameters across geographical areas, species and time periods. For instance, a comparison of some blood parameters related to cell components of the immune response, plasmatic carbohydrates, and lipids and proteins as indicators of nutrition was reported for Red Knots *Calidris canutus rufa* along their northward Atlantic migratory route (D'Amico 2009).

For migratory shorebird species in South America, values of physiological traits remain scarce in the literature. This is particularly true for reference ranges of blood variables in shorebirds in Argentina. Data exist only for Red Knots at Rio Negro and Tierra del Fuego provinces (D'Amico *et al.* 2010). However, the whole Patagonian region is important for the conservation of migrant shorebirds.

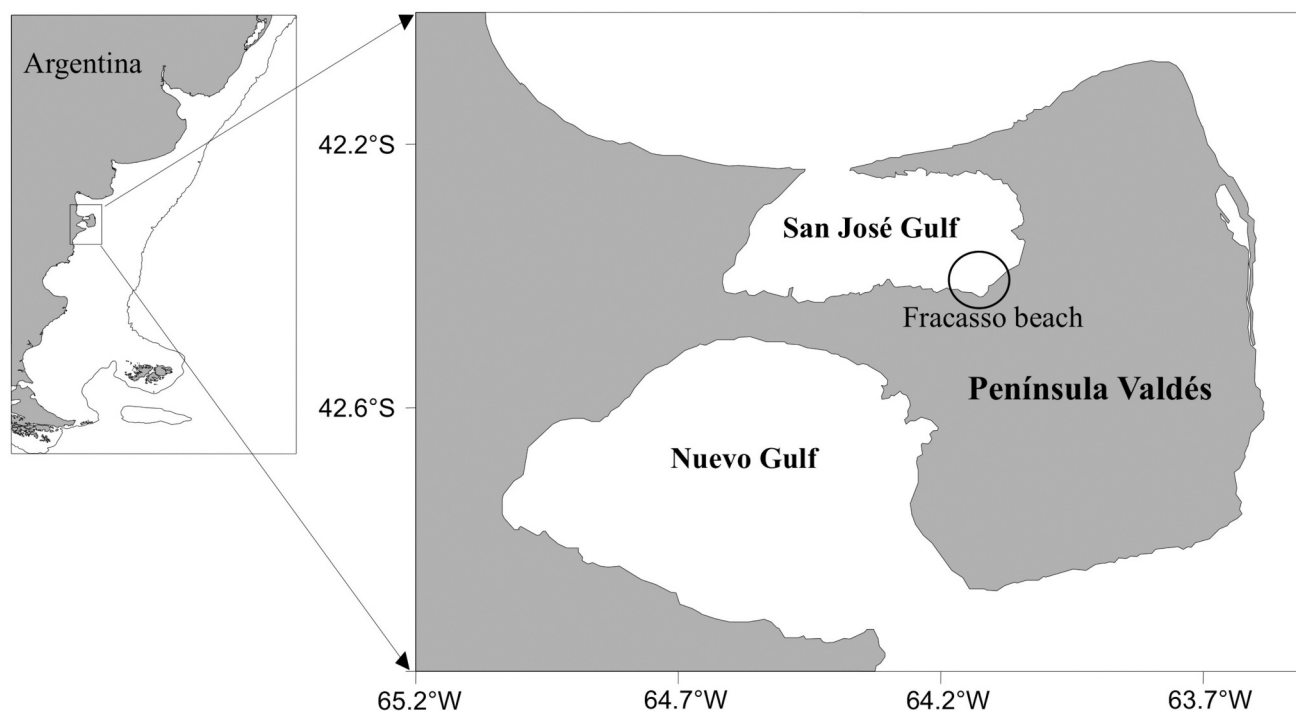
Patagonia contains concentrations of key habitats for non-breeding populations of long-distance Nearctic species

such as Red Knots and White-rumped Sandpipers *Calidris fuscicollis* (Morrison & Ross 1989), as well as habitats for short-distance Neotropical species such as Two-banded Plovers *Charadrius falklandicus*. Some of these sites in Patagonia are key areas for endangered and high conservation priority species such as Red Knots (Niles *et al.* 2008, Gratto-Trevor *et al.* 2011). One such site is Peninsula Valdés (Chubut) Argentina, which the Western Hemisphere Shorebird Reserve Network has recently nominated as a site of Regional Importance.

Here we present, for the first time, a report of blood parameters related to the body condition and the immune function of Red Knots, White-rumped Sandpipers and Two-banded Plovers captured at Peninsula Valdés, Patagonia, Argentina, during the non-breeding season.

## METHODS

Birds were captured at Fracasso Beach, Peninsula Valdés (Fig. 1) during their non-breeding season during March and April in 2011 and 2016. We used cannon nets to capture birds roosting at high tide following standard protocols (Kasprzyk & Harrington 1989). All captured individuals were kept in shaded cages placed on the sand and the cages and sand were continuously moistened to avoid the birds overheating before they were sampled (Kasprzyk & Harrington 1989). Birds of all species were taken from the shaded cages at random for sampling. The time that elapsed between capture and blood draw was recorded for all individuals in 2016 and ranged from 10 to 110 min.



**Fig. 1.** Location of the study site at Fracasso Beach, Península Valdés, Patagonia, Argentina.

Each bird was weighed with an analytical balance ( $\pm 0.01$  g). Blood samples (0.3–0.4 ml) were extracted from the brachial vein using 27G needles and collected into microcapillary tubes (Tecnon, Argentina). Blood was then stored at 4°C until centrifugation and analysis in the lab. Blood smears were prepared with a drop of fresh blood, air-dried, fixed with absolute ethanol for 3 min and stained with *Tinción 15* (Biopur S.R.L., Rosario, Argentina). All Red Knots and White-rumped Sandpipers were considered adults based on their plumage and molt characteristics, and for Two-banded Plovers, individuals were considered adults based on the two distinguishable breast bands following Narosky & Yzurieta (2010) and/or their body mass (range 62–72 g; Wiersma *et al.* 2016). All individuals sampled were deemed to be in good health with no signs of illness and no signs of feather damage from ectoparasites. Birds were released at the site of capture after sampling.

Once in the laboratory, we centrifuged tubes containing extracted blood at 12,000 g for 12 min (Cavour VT 1224, Argentina) and hematocrit was measured with a micro-hematocrit ruler. Hematocrit is considered an index of general condition and estimator of the aerobic capacity (Fair *et al.* 2007, Beldomenico *et al.* 2008).

To access the current nutritional status of birds, blood carbohydrates lipids and total proteins were measured. Glucose, triglycerides and cholesterol were obtained *in situ* using specific Accutrend test strips and performed in Accutrend Plus (Roche Diagnostics, Switzerland). This method is useful for small blood volumes like those obtained in this study. This method has been previously validated using a spectrophotometer (Metrolab 1600 Plus,

Argentina) in penguins (D'Amico *et al.* 2016). Total proteins were analyzed by colorimetric methods and processed on a spectrophotometer (Metrolab 1600 Plus, UV-Vis, Argentina).

Blood smears were examined under a light microscope scanning monolayer fields with similar densities of erythrocytes for all individuals to obtain the white blood cell counts as one of the components of the immune system, the primary defence against pathogens in animals (Campbell 1995). To provide information on the cellular components of the immune system, total white blood cell count per 10,000 erythrocytes was estimated by counting the number of erythrocytes in one microscopic visual field and multiplying it by the number of microscopic visual fields that were scanned until reaching 100 leukocytes (Lobato *et al.* 2005). The proportion of each leukocyte type was obtained from a sample of 100 leukocytes under 1000x (oil immersion) classified into basophils, heterophils, eosinophils, lymphocytes, and monocytes (Campbell 1995). Total counts for each leukocyte were obtained by multiplying the total leukocyte count and the respective percentage. Heterophil/lymphocyte ratio (H/L), a measure of stress (Davis *et al.* 2008), was calculated from the corresponding leukocyte counts. For the three species, eight smears were randomly selected for analysis of repeatability. Three consecutive leukocyte counts were made using the described counting method for each of the eight smears. Repeatability was calculated following Lessells & Boag (1987) for total leukocyte counts (TLC), heterophils (H), eosinophils (E), lymphocytes (L) and monocytes (M). Basophils (B) were not included because of many zeros in the matrix. All values

of repeatability were between 0.74 and 0.98. All leukocyte counts were made by VLD.

Statistical analyses were performed using STATISTICA version 7.0 and statistical significance is reported using an alpha of 0.05. Data from the different years (2011:  $n = 19$ , 9 White-rumped Sandpipers and 10 Red Knots; 2016:  $n = 48$ , 35 Two-banded Plovers, 8 White-rumped Sandpipers and 5 Red Knots) were pooled to perform further analyses since none of the variables showed significant differences between years (Mann-Whitney  $U$ -test, all  $P > 0.05$ ). In order to test if physiological parameters were affected by handling time we used Spearman R correlations (Sokal & Rohlf 2012). Physiological parameters obtained were

compared between the two Nearctic, and Nearctic *versus* Neotropical species using the nonparametric Mann-Whitney  $U$ -test. Parameters were also compared among the three species using Kruskal-Wallis  $H$ -test.

## RESULTS AND DISCUSSION

A total of 67 individual birds were sampled and released. All physiological values, as well as handling time after capture (for 2016), are shown in Table 1 for Red Knots, White-rumped Sandpipers and Two-banded Plovers.

Handling time (time elapsed between capture and sampling) was recorded in 2016 and ranged from 10 to 110 min.

**Table 1.** Parameters obtained for Red Knots, White-rumped Sandpipers and Two-banded Plovers in Fracasso beach, Peninsula Valdés, Argentina during the non-breeding season. Values provided are the mean and standard error (SE), median and range of minimum and maximum of data obtained. Hto = hematocrit, GLU = glucose, TGL = triglycerides, CHOL = cholesterol, TP = total proteins, WBC = white blood cell counts, H/L = heterophil-lymphocyte ratio, % = percentages and t = total counts of B (basophils), E (eosinophils), H (heterophils), L (lymphocytes), M (monocytes) and HTime (handling time).

Parameter	Red Knot ( $n = 15$ )		White-rumped Sandpiper ( $n = 17$ )		Two-banded Plover ( $n = 35$ )	
	Mean $\pm$ SE	Median (min-max)	Mean $\pm$ SE	Median (min-max)	Mean $\pm$ SE	Median (min-max)
Body mass (g)	124.4 $\pm$ 1.7	125 (110-135)	44.4 $\pm$ 0.6	44 (41-50)	63 $\pm$ 0.6	63 (56-69)
Hto (%)	51.9 $\pm$ 1.09	52 (46-58)	52.8 $\pm$ 0.5	53 (50-56)	51.5 $\pm$ 0.4	52 (46-58)
GLU (mg/dl)	355.6 $\pm$ 13.01	362 (230-420)	310.4 $\pm$ 8.7	313.5 (233-370)	260 $\pm$ 8.2	260 (155-375)
TGL (mg/dl)	103.2 $\pm$ 9.6	91 (70-206)	123.4 $\pm$ 19.9	92.5 (75-384)	101.2 $\pm$ 9	89 (65-352)
COL (mg/dl)	246.4 $\pm$ 15.7	253 (100-320)	229.5 $\pm$ 12.2	215 (166-300)	224.2 $\pm$ 6.7	220 (145-305)
TP (g/dl)	6.7 $\pm$ 0.1	7 (6-7.2)	5.5 $\pm$ 0.1	5.5 (5-6.4)	5.6 $\pm$ 0.09	5.5 (4.5-6.8)
WBC	34 $\pm$ 1.1	34 (25-40)	27 $\pm$ 1.3	26 (19-36)	38.2 $\pm$ 2.9	32 (12-86)
H/L	0.5 $\pm$ 0.02	0.5 (0.4-0.7)	0.8 $\pm$ 0.1	0.6 (0-2)	0.7 $\pm$ 0.05	0.6 (0.3-1.6)
%B	0.7 $\pm$ 0.2	1 (0-2)	2.8 $\pm$ 0.3	3 (0-5)	0.5 $\pm$ 0.1	0 (0-5)
%E	3.6 $\pm$ 0.3	4 (2-7)	2.5 $\pm$ 0.4	2 (0-7)	18.4 $\pm$ 1.1	17 (7-35)
%H	30.4 $\pm$ 0.8	31 (25-36)	39.1 $\pm$ 2.8	36 (24-60)	31.7 $\pm$ 1.8	30 (15-61)
%L	60.4 $\pm$ 0.9	60 (54-67)	51.1 $\pm$ 2.9	54 (30-67)	48.8 $\pm$ 1.6	50 (32-65)
%M	4.8 $\pm$ 0.3	5 (3-7)	4.2 $\pm$ 0.3	4.5 (2-6)	6.2 $\pm$ 0.4	6 (2-16)
tB	26.3 $\pm$ 7.5	31 (0-78)	76.2 $\pm$ 11	69 (0-170)	17.4 $\pm$ 5.8	0 (0-180)
tE	121 $\pm$ 14.03	117 (54-275)	71.7 $\pm$ 14.6	45 (0-178)	723.6 $\pm$ 80.3	589 (133-2150)
tH	1037.8 $\pm$ 49.02	1054 (631-1360)	623.1 $\pm$ 133.9	745.5 (45-1350)	1083 $\pm$ 174.7	1000 (43-5246)
tL	2051.3 $\pm$ 73.83	2052 (1641-2600)	1358.8 $\pm$ 94.6	1264 (897-2423)	1878.8 $\pm$ 156.8	1467 (384-4030)
tM	162.6 $\pm$ 12.97	165 (93-252)	115 $\pm$ 11.2	106.5 (48-192)	241 $\pm$ 26.7	190 (36-630)
HTime* (min)	40 $\pm$ 10	38 (21-62)	96 $\pm$ 10	96 (82-110)	38 $\pm$ 10	38 (10-78)

\*Handling time after capture was only recorded in 2016; therefore samples sizes are: Red Knot = 5, White-rumped Sandpiper = 8 and Two-banded Plover = 35.

Although we sampled birds of all species at random (birds were taken from keeping cages for sampling at random) handling time differed among species ( $H$ -test,  $P = 0.0001$ ). Only two of the physiological parameters measured showed significant changes with handling time: TLC and H/L. Two-banded Plovers displayed decreased values of TLC after 41 min since capture ( $R = -0.34$ ,  $P = 0.040$ ), and Red Knots had increased H/L values after 51 min since capture ( $R = 0.91$ ,  $P = 0.028$ ). In contrast, no blood parameters showed changes with handling time in White-rumped Sandpipers. Decreases in TLC values relative to time since capture have previously been reported in shorebirds. For example Buehler *et al.* (2008a) reported that TLC values decreased within 1 h of capture in Red Knots. H/L values are known to increase due to several stressful situations such as changes in nutrition (Davis *et al.* 2000), parasitic infection (Lobato *et al.* 2005) and migration (Owen & Moore 2006). Buehler *et al.* (2008a) found a significant decrease in lymphocytes and a trend towards an increase in heterophils 60–90 min after capture in Red Knots.

Values of hematocrit ranged between 46% and 58%. The average for Red Knots was 51.9%, for White-rumped Sandpipers 52.8%, and for Two-banded Plovers 51.5% (Table 1). In general, reference ranges of hematocrit reported for birds are 35–55% (Campbell 1995). However, migrant birds usually exhibit wider ranges because of the high aerobic requirements of migratory flights (Carpenter 1975, Wingfield *et al.* 1990). Values of hematocrit obtained did not show significant differences among the three species in this study ( $H$ -test,  $P = 0.32$ ) and they were in accord with those reported for other migrant shorebird species (Piersma & Everaarts 1996, Jenni *et al.* 2006). There were no statistically significant differences in haematocrit between the two Nearctic species ( $U$ -test,  $P = 0.64$ ) or between the Nearctic and Neotropical species ( $U$ -test,  $P = 0.19$ ).

Values of glucose and total proteins showed statistically significant differences among species ( $H$ -test,  $P < 0.0001$  for both parameters), and they were higher for Nearctic species – Red Knots and White rumped-Sandpipers – than for the Neotropical Two-banded Plover ( $U$ -test,  $P < 0.0001$  and  $P = 0.008$  for glucose and total proteins respectively). Between Nearctic species, Red Knots showed higher values of glucose ( $U$ -test,  $P = 0.005$ ) and total proteins ( $U$ -test,  $P < 0.0001$ ) than White-rumped Sandpipers (Table 1). These differences in biochemical levels, could be related to the prey items they feed on at the site. The diet of Red Knots and White rumped Sandpipers is mostly the clam *Darina solenoides*, the two species taking different sizes according to their bill lengths: 8–26 mm for Red Knots (Musmeci *et al.* 2015) and 1–8 mm for White-rumped Sandpipers (D'Amico *et al.* 2004). The main prey of Two-banded Plovers in terms of energy intake is the polychaete *Glycera americana* (D'Amico & Bala 2004).

For the three species, lymphocytes were the most abundant type of leukocyte followed by heterophils (Table 1), as is common for birds (Campbell 1995). White-rumped Sandpipers showed higher values of % heterophils compared

to Red Knots ( $U$ -test,  $P = 0.017$ ) and consequently higher values of H/L ratio ( $U$ -test,  $P = 0.034$ ). White-rumped Sandpipers also showed higher values of % basophils ( $U$ -test,  $P < 0.0001$ ) and total basophils ( $U$ -test,  $P = 0.002$ ). However, Red Knots showed higher values of TLC ( $U$ -test,  $P = 0.002$ ), % lymphocytes ( $U$ -test,  $P = 0.016$ ), total lymphocytes ( $U$ -test,  $P < 0.0001$ ), total eosinophils ( $U$ -test,  $P = 0.034$ ), and total monocytes ( $U$ -test,  $P = 0.036$ ) than White-rumped Sandpipers. Average values of H/L ratios were  $< 1$  for the three species (Table 1).

Two-banded Plovers showed the highest values of eosinophils ( $H$ -test,  $P < 0.0001$ , Table 1). Although there can be some inter-species variation, in general eosinophils, basophils and monocytes are present in low percentages in healthy birds (Campbell 1995). Although eosinophils can be produced in response to infections (Campbell 1995), their increased values are usually related to gastrointestinal parasitic infections (Thrall *et al.* 2012). In this study, we did not include endoparasitic loads because of the requirement of dead specimens for testing. Thus we do not know whether the Two-banded Plovers we sampled had gastrointestinal parasitic infections or whether Two-banded Plovers display higher values of eosinophils even when not ill. The birds we sampled did not show external signs of illness.

Physiological parameters can change over the annual cycle. For instance, total leucocyte counts and heterophil and lymphocyte counts varied significantly over the annual cycle in Red Knots (Buehler *et al.* 2008b). Therefore some of the differences we found could be associated with the stage of the annual cycle. Although we sampled all species at a similar time of year (March–April), long-distance migrants have to prepare for flight to reach their reproductive areas, whereas Two-banded Plovers have a wider window to get into optimal condition for reproduction, which sometimes takes place on the beaches of Peninsula Valdés. Thus, parameters related to nutrition, such as higher values of glucose and total proteins found for Nearctic compared to the Neotropical species, could be associated with the stage of the annual cycle.

The blood parameters measured in this study represent a spectrum of hematologic findings for apparently healthy shorebirds at Peninsula Valdés, Argentina, during the non-breeding season. Although the sample size in this study is small and we were unable to obtain information on the sexes of the birds sampled, this work provides some baseline data on blood parameters at an important site for shorebirds in Argentina.

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