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Hematologic and Plasma Biochemistry Values for Endangered Red Knots (*Calidris canutus rufa*) at Wintering and Migratory Sites in Argentina

Verónica L. D'Amico,[1,4] Marcelo Bertellotti,[1] Allan J. Baker,[2] and Patricia M. González[1,3] [1] Centro Nacional Patagónico, Bvard. Brown 2825, Puerto Madryn CP 9120, Chubut, Argentina; [2] Department of Natural History, Royal Ontario Museum, Toronto, Canada; [3] Fundación Inalafquen, San Antonio Oeste, Río Negro, Argentina and Global Flyway Network; [4] Corresponding author (email: damico@cenpat.edu.ar)

ABSTRACT: We obtained hematologic and plasma biochemistry values for adult, long-distance migrant Red Knots at their southernmost wintering site in Río Grande (Tierra del Fuego, Argentina) and at the first stopover site in San Antonio Oeste (Río Negro, Argentina). Lymphocytes (L) followed by heterophils (H) were the most abundant leukocytes. H/L ratio and glucose levels were significantly higher at Río Grande, possibly because of the stress of migration and molting. Packed cell volume results ranged widely, probably in response to increased oxygen demand for migration. Protein profiles and lipids were higher at the stopover site and attributable to birds storing reserves for subsequent flights.

Key words: Hematologic biochemistry values, plasma biochemistry values, Red Knots, stopover sites, wintering sites.

Red Knots (*Calidris canutus rufa*, Scolopacidae) are long-distance migrants that breed in the Arctic tundra and migrate south to coastal wintering sites in Tierra del Fuego (TDF), northern Brazil, or Florida (Baker et al., 2005). The TDF population flies about 30,000 km annually on the return trip to the Arctic. These migrations are metabolically demanding and entail changes in plasma biochemistry and hematology (Brown, 1996; Piersma et al., 1996; Jenni et al., 2006) that vary across environments (Artacho et al., 2007). The TDF population of Red Knots has declined from 51,000 in 2000 to around 14,900 in 2008 (Morrison et al., 2004; Morrison, 2008), mainly because of a decline in the food supply at the last stopover site in Delaware Bay (USA) before departing for the breeding grounds in the Arctic (Baker et al., 2004). Consequently the subspecies *rufa* has been recommended for listing as endangered in Canada (COSEWIC, 2007).

Plasma biochemistry and hematology are good methods to evaluate physical condition in wild birds (Brown, 1996), but values for wild migratory shorebird species in the Americas are scarce in the literature. We present hematologic and plasma biochemistry values for long-distance migrant Red Knots from two Argentine sites: the southernmost wintering site at Río Grande, Tierra del Fuego (67.5°W, 53.4°S), and at the first stopover site during the following northward migration at San Antonio Oeste, Río Negro (64.5°W, 40.5°S). Birds were sampled at Río Grande soon after their arrival in November 2005 and in March 2006 at San Antonio Oeste.

Birds were captured with cannon nets, and all individuals sampled were adults (3 yr old or older). All individuals were assumed to be in good health because previous collections at this site showed the Red Knots from these sites had no external signs of illness based on the brightness of plumage, good flight capacity, absence of feather damage from ectoparasites, and negative tests for some infectious agents (D'Amico et al., 2007). Each bird was weighed with an analytical balance to the nearest 0.01 g. Blood samples (0.3–0.6 ml) were extracted from the brachial vein using 0.5×15 mm needles and collected into heparinized microcapillary tubes (ML0067 40 mm SafeCrit, MarketLab, Caledonia, Minnesota, USA), which were stored at 4 C for up to 3 h. Thin smears were prepared from fresh nonheparinized blood on individual slides, air dried, fixed with methanol for 3 min and stained with Giemsa (Merck, Darm-

TABLE 1. Mean±SE and range (minimum–maximum) of leukocyte profile, heterophil/lymphocyte ratio (H/L), and packed cell volume or hematocrit (PCV) values for Red Knots from two sites in Argentina. Sample size (*n*) is shown in parentheses. H=heterophils; L=lymphocytes; M=monocytes; B=basophils, E=eosinophils.

	Río Grande		San Antonio Oeste	
	Mean±SE	Range	Mean±SE	Range
H (%)	40.6±1.79 * (*n*=96)	4–75	21.3±2.02* (*n*=34)	6–61
L (%)	49.8±1.8* (*n*=96)	21–87	64.49±2.9* (*n*=34)	30–88
M (%)	6.4±0.34* (*n*=96)	0.9–14	9.24±0.88* (*n*=34)	2–24
B (%)	2.5±0.23* (*n*=96)	0–6	1±0.23* (*n*=34)	0–4
E (%)	0.4±0.08 (*n*=96)	0–4	0.95±0.3 (*n*=34)	0–8
H/L	1.08±0.08* (*n*=96)	0.04–3.5	0.40±0.07* (*n*=34)	0.06–0.4
PCV (%)	51.3±0.34 (*n*=96)	36–59	51.2±0.87 (*n*=55)	32–65

* Significant difference between sites ($P<0.003$).

stadt, Germany). Tubes were centrifuged at 13,700 × G for 2 min (CritSpin Haematocrit Centrifuge model M961) to separate cellular and plasma components. Hematocrit or packed cell volume (PCV) was measured with a microhematocrit ruler (J. P. Selecta, Abrera, Spain). Blood smears were examined under oil immersion (1,000×), and a sample of 100 leukocytes was classified into basophils, heterophils, eosinophils, lymphocytes, and monocytes (Campbell, 1994; Hale and Briskie, 2007). The heterophil/lymphocyte ratio (H/L) was used as an indicator of stress in birds (Gross and Siegel, 1983; Maxwell and Robertson, 1998).

Plasma for biochemical analyses was processed on a spectrophotometer (METROLAB 1600 Plus, UV-Vis Metrolab, Bernal, Buenos Aires, Argentina) to determine total proteins (TP; g/dl), albumin (ALB; g/dl), globulins (GLOB; g/dl), ratio of albumin/globulins (A/G), cholesterol (CHOL; mg/dl), triglycerides (TGL; mg/dl), and glucose (GLU; mg/dl).

Hematology and plasma biochemistry values were compared between the sampled sites using the nonparametric Mann-Whitney *U* test (STATISTICA version 6.0, StatSoft, Tulsa, Oklahoma, USA) because of the lack of normality and homoscedasticity of the data (Sokal and Rohlf, 1995). To maintain an experiment-wise error rate of 0.05, we used a sequential Bonferroni adjustment (Rice, 1989) of $\alpha=0.003$ for $n=15$ parameters compared, including hematology, plasma biochemistry, and weight.

Lymphocytes were the most abundant type of leukocyte followed by heterophils (Table 1), as observed in other long-distance migratory birds, including the *islandica* subspecies of Red Knots (Owen and Moore, 2006; Buehler, 2008). Lymphocytes and monocytes were significantly lower after arrival at the wintering site in Río Grande ($P<0.003$), probably because the birds are energetically stressed and possibly immunocompromised after completing the long southern migration (Owen and Moore, 2006). In addition, Buehler (2008) found that Red Knots in Delaware Bay (USA) had lower lymphocytes and monocytes during the recovery period after arrival than during the subsequent fuel storage period.

Soon after arrival at the southern wintering site at Río Grande the H/L ratios were significantly higher than at San Antonio Oeste ($P<0.003$) as a consequence of an elevated proportion of heterophils ($P<0.003$). This increase could result from the cumulative stress induced by the long southern migration (Owen and Moore, 2006). Another explanation could be injury to active skeletal muscle fibers during long flights, because heterophils and other phagocytic cells

TABLE 2. Mean±SE and range (minimum–maximum) of plasma biochemistry values and weight (WGT) of Red Knots from two sites in Argentina. Sample size (n) is shown in parentheses. TP=total proteins (g/dl); ALB=albumin (g/dl); GLOB=globulins (g/dl); A/G=albumin/globulins; CHOL=cholesterol (mg/dl); TGL=triglycerides (mg/dl); GLU=glucose (mg/dl).

	Río Grande		San Antonio Oeste	
	Mean±SE	Range	Mean±SE	Range
TP (g/dl)	3.49±0.05* (n=96)	2.5–5	3.76±0.06* (n=55)	2.33–4.43
ALB (g/dl)	2.01±0.03* (n=96)	1.3–3.1	1.78±0.04* (n=55)	1.26–2.17
GLOB (g/dl)	1.48±0.04* (n=96)	0.5–2.4	1.98±0.05* (n=55)	0.77–2.81
A/G	1.48±0.06* (n=96)	0.63–4.4	0.94±0.04* (n=55)	0.48–2.03
CHOL (mg/dl)	206.58±3.81* (n=96)	123–302	291.8±8.05* (n=47)	123–384
TGL (mg/dl)	96.88±2.27* (n=96)	54–178	177.2±5.35* (n=23)	113–212
GLU (mg/dl)	323.14±4.71* (n=96)	228–450	200.6±4.42* (n=13)	181–225
Weight (g)	125.3±1.02 (n=96)	106–150	119±2.03 (n=29)	100–140

* Significant difference between sites (P<0.003).

such as basophils were also significantly higher at Río Grande (Smith, 1991). Birds arriving at Río Grande usually begin energetically expensive feather molt, which might also contribute to elevated H/L ratios at that site (González, 2007).

Values of PCV were similar between sites (P>0.003, Table 1). Nevertheless, compared to the ranges for the *islandica* subspecies of Red Knots captured in the Dutch Wadden Sea (mean=48, range=40–55; Piersma et al., 2000), the values in this study were a little higher. Higher PCV in the *rufa* subspecies might reflect greater oxygen transport needs for their longer migration.

The significantly elevated TP, TGL, and CHOL at the San Antonio Oeste stopover (P<0.003, Table 2) probably reflect mobilization of stored lipids and proteins during the migratory flight from the wintering site in Río Grande, as well as storage of nutrients in some of the heavier refueling birds (Landys et al., 2005; Jenni-Eiermann et al., 2009). Carbohydrates are the most important energy source in migrant birds not involved in intensive activity such as migration (Millar et al., 1984; Berthold, 1996). Therefore, the significantly higher values of GLU in birds at the wintering site Río Grande (P<0.003, Table 2) compared to migrant birds refueling at San Antonio Oeste is consistent with these expectations. It also has been proposed that fat levels could be related to a switch in peripheral catabolism to the oxidation of fats resulting in lower glucose utilization, which may be important for birds feeding at stopover sites, especially in view of fasting during long migratory flights (Totzke et al., 1998).

Capture stress could also have altered some of these parameters (Romero and Romero, 2002; Lynn and Porter, 2008); however, we attempted to minimize this by processing blood samples within 3 hr of collection (Dietz et al., 2009). One limitation of this study is the lack of data on sex and age of individuals. In addition, we were unable to capture birds more than once at each site because of restrictions for working with an endangered species. Despite the possible interactive effects of the physiological stress of migration and capture, the hematologic values for birds in this study could be viewed as representing a spectrum of hematologic findings for apparently healthy Red Knots.

Future efforts should focus on continued monitoring of body condition and health studies because they are a vital component of any population recovery plan. The conservation of wintering and feeding habitats along the flyway also needs to be a priority.

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