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Hematology, Biochemistry and Serum Protein Analyses of Antarctic and non-Antarctic Skuas

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Abstract.—Determination of hematological and biochemical parameters provides important data to assess the physiological condition in wild birds. Therefore, to carry out ecophysiology or conservation studies it is essential to establish baseline physiological parameters and how these change with age and life history events. Hematological (hematocrit, hemoglobin and erythrocyte sedimentation) and biochemical (glucose, total lipids and proteins, aspartate and alanine aminotransferase activities and electrolyte concentration) reference values were determined in two Antarctic migratory bird species, the Brown Skua (*Stercorarius antarcticus*) and South Polar Skua (*S. maccormicki*), from South Shetland Island during breeding season. Also, hematological data (hematocrit) were determined for non-Antarctic skuas, with Chilean (*S. chilensis*) and Falkland (*S. antarcticus antarcticus*) skuas sampled in the Beagle Channel islands (Tierra del Fuego Province) and Viana Island (Chubut Province), Argentina, respectively. Differences between adult Antarctic skua species were observed in hemoglobin, erythrocyte sedimentation, total lipids and aspartate aminotransferase activity. In addition, age-related differences in Antarctic skuas in hematocrit, hemoglobin, glucose and total protein values were observed. Serum reference protein fractions (Albumin, α_1 , α_2 , β and γ globulins) were assessed by electrophoresis for Antarctic and non-Antarctic skuas. Similar protein patterns were observed between South Polar and Chilean skuas as well between Falkland Skua and Brown Skua. The differences between adult sympatric Antarctic skuas may be related to their nutritional status and species-specific migrations, feeding habits and the differential use of the breeding niches, while the age variation may be related to physiological development processes in chicks or to the energy expenditure in adults during breeding. Received 28 June 2014, accepted 14 January 2015.

Key words.—Antarctic, biochemistry, hematology, protein electrophoresis, skuas.

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Determination of hematological and biochemical parameters offers an efficient method to assess body condition in free living birds. These parameters reflect the momentary metabolic state, but also are influenced by different seasonal processes related to molting, breeding and migratory behavior (Jenni-Eiermann *et al.* 2002). Moreover, increasing evidence indicates that specific physiological responses throughout the life cycle may be taxon specific (Cooke *et al.* 2013). Migratory birds are exposed to extreme metabolic demands. Coupled with other factors such as stress situations, physical activity, quality and availability of food resources or environmental contaminants are able to induce alterations in blood parameters (Sturkie and Griminger 1986; Vleck and Vleck 2002), that then may lead to migratory and breeding constraints (Studds and Marra 2005).

Antarctic birds are important members of the Antarctic ecosystem in terms of total bio-

mass and interaction with the environment (Corsolini 2011). In particular, Brown Skua (*Stercorarius antarcticus*) and South Polar Skua (*S. maccormicki*) (hereafter Antarctic skuas) are top predators that nest in subantarctic islands and the Antarctic (Ritz *et al.* 2006, 2008). When breeding in sympatry, Brown Skuas monopolize the terrestrial feeding resources (i.e., penguin colonies) while South Polar Skuas feed at sea (i.e., fish) (Montalti *et al.* 2009; Graña Grilli and Montalti 2014). The skuas that feed at sea have wider home ranges and take longer feeding trips, leaving their nests unattended for longer periods than those feeding on terrestrial resources and searching for food near their nests (Graña Grilli *et al.* 2011). After the breeding season, South Polar Skuas migrate to northern localities (near Greenland, Japan and Canada (Furness 1987; Kopp *et al.* 2011). Brown Skuas reach the Atlantic Ocean and Pacific Ocean tropics (Phillips *et al.* 2007).

The breeding sites of Chilean Skuas (*S. chilensis*) comprise the islands and channels that lie south and west of Tierra del Fuego and the southern Andes (Furness 1987), while the breeding sites of Falkland Skuas (*Stercorarius antarcticus antarcticus*) occur along the Atlantic coast of Patagonia. Chilean and Falkland skuas (hereafter non-Antarctic skuas) are sympatric in the area north of the Santa Cruz Province (Devillers 1978). Due to overlap of some breeding areas, hybridization between South Polar and Brown skuas occurs (Ritz *et al.* 2006), and mixed pairs between South Polar and Chilean skuas have been recorded in Antarctica (Reinhardt *et al.* 1997). Evaluation of these features is important for determining how these species adapt to different environments to ensure better management of resources and to understand the population dynamics.

There is little reported data about normal hematology and biochemistry of Antarctic skuas (Milsom *et al.* 1973; Myrcha and Kostelecka-Myrcha 1980; Rosa *et al.* 1993; Kursa and Bezrukov 2008), while no studies have been reported on non-Antarctic skuas. There were no reported comparisons about hematological and biochemical reference values between adults and chicks of these species.

The aim of this study was to establish baseline hematological and biochemical values for Antarctic skuas during the breeding season. In addition, we identified hematological parameters (only hematocrit) for two non-Antarctic skua species, the Chilean and Falkland skuas, sampled during the breeding season. Finally, we performed an electrophoretic analysis of serum proteins to characterize and document the normal reference range and species-specific electrophoretic fractions.

METHODS

Study Area

The present study was conducted at the Potter peninsula in King George Island, South Shetland Islands, Antarctica (62° 15' S, 58° 40' W) (Fig. 1), where both Antarctic skua species breed sympatrically. This work

was conducted from November 2009 to February 2010. At that time, a total of 77 breeding pairs (Brown Skuas: $n = 35$ pairs; South Polar Skuas: $n = 42$ pairs) were present in the Potter peninsula. Chilean Skua blood samples were obtained in the Beagle Channel islands, Tierra del Fuego Province, Argentina, in February 2010, and Falkland Skuas were sampled in December 2009 on the Viana Island, Chubut Province, Argentina (Fig. 1).

Sample Collection

Skuas were captured using a rope with a snare trap. A total of 44 Brown Skuas (adults: $n = 32$; chicks: $n = 12$), 29 South Polar Skuas (adults: $n = 20$; chicks: $n = 9$), four Chilean Skuas ($n = 4$ chicks) and six Falkland Skuas ($n = 6$ adults) were sampled independently from sex. Chicks sampled were between 10-20 days old. Peripheral blood was extracted by the brachial vein using a 22-gauge needle within 3 min after birds were captured to avoid changes in biochemical and hematological parameters due to handling associated stress. To obtain serum, blood samples were incubated at room temperature for 3 hr and then overnight at 4 °C. After that, clotted blood was centrifuged at 8,000 rpm for 10 min and serum was harvested. Finally, samples were stored at -20 °C until laboratory analyses were performed. In some cases, blood volumes obtained were small; therefore, for some samples, analyses of all the parameters could not be performed.

Hematological and Biochemical Analysis

Different blood parameters were determined: hematocrit, hemoglobin, erythrocyte sedimentation, metabolites associated with metabolism (glucose, total lipid and proteins), aspartate and alanine aminotransferase activity (AST and ALT) and concentration of electrolytes (Na^+ , K^+ and Cl^-) to assess dehydration. Only hematocrit was determined in non-Antarctic skua species. To calculate hematocrit, blood (500 μl) was stored in a heparinized capillary tube, and the sample was centrifuged at 5,000 rpm for 20 min. Hemoglobin concentration was measured by adding 10 μl of well-mixed blood to 5 ml of Drabkin's reagent. After 10 min, this was centrifuged at 2,500 rpm for 5 min to avoid interference of lysed nuclei during the determination of the optical density, which was measured in a spectrophotometer set at 540 nm (Drabkin and Austin 1935; Hawk and Oser 1965). The standard sedimentation value of erythrocytes was calculated using 2 ml of heparinized blood in a Westergreen pipett for 15, 30 and 60 min.

Glucose concentration was estimated using the orthotoluidine method in a spectrophotometer set at 505 nm (Hyvarinen and Nikkila 1962). Lipid concentration was determined using the sulfo-phospho-vanillin method in a spectrophotometer set at 510 nm (Frings and Dunn 1970). Total plasma protein concentration was assessed using a previously described chemical method (Wesichselbaum 1949). Also, plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using a colorimetric method (Reitman and Frankel 1957). Sodium, potassium and chloride concentrations were determined using a selective ion analyzer (EasyLyte).

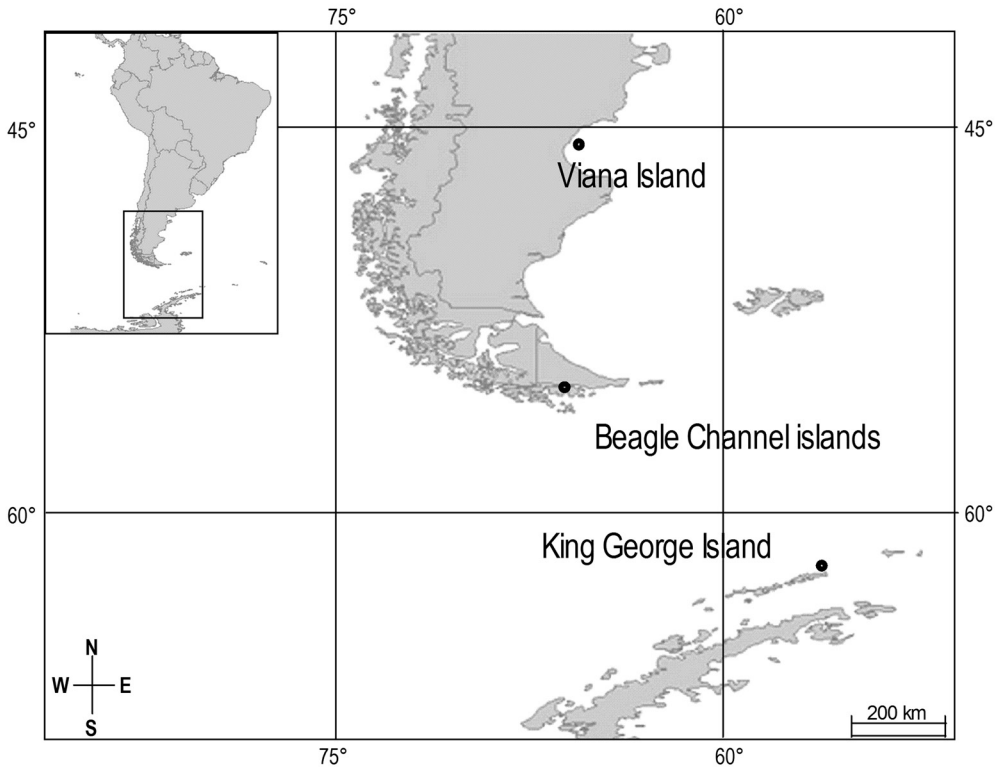


Figure 1. Study sites located on King George Island (South Shetland Islands, Antarctica); Beagle Channel Islands (Tierra del Fuego Province, Argentina) and Viana Island (Chubut Province, Argentina).

Polyacrylamide Native Gel Electrophoresis

The serum protein profile was examined using a 10% native polyacrylamide gel (Laemmli 1970). Serum was diluted in sample buffer at a 1:10 ratio. A molecular weight standard was prepared by mixing proteins of known molecular weights (M_w 272, 132, 45, 29 and 14 kDa). Samples were then assayed by a polyacrylamide gel electrophoresis using a vertical electrophoretic plate (Hoffer). After that, gels were stained with 0.25% Coomassie Brilliant Blue R250 and discolored with a solution containing 10% acetic acid and 10% methanol. After electrophoretic separation, serum protein pattern was determined by densitometric analyses (Image-Pro Plus, Inc. 2013), and relative abundance of each fraction was calculated.

Statistical Analysis

Statistical analysis and plotting were performed using GraphPad Software, Inc. (2007). Normality and homogeneity of variance were tested using Kolmogorov-Smirnov and Levene tests. All compared data were normally distributed and t -test (two-tailed) was used to examine differences in hematological and biochemical parameters between species and ages. Results with a P -value less than 0.05 were considered significantly dif-

ferent. Results were expressed as mean \pm SD for each species, and values of t and df, as t_{df} , were described for each hematological and biochemical parameter.

RESULTS

Hematology

We determined hematocrit values for Antarctic skuas (adults and chicks) (Fig. 2A). No differences were observed in hematocrit values between Brown and South Polar skua adults (t -test $t_{50} = 1.399$, $P > 0.05$). However, hematocrit values were higher in adults than in chicks for both Antarctic skua species (Brown Skua: t -test $t_{42} = 11.42$, $P < 0.001$; South Polar Skua: t -test $t_{27} = 9.641$, $P < 0.001$). In addition, we assessed hematocrit value in non-Antarctic skuas. No differences were observed among adult Antarctic and non-Antarctic species (Falkland Skua vs. Brown Skua: t -test $t_{36} = 0.1184$, $P > 0.05$;

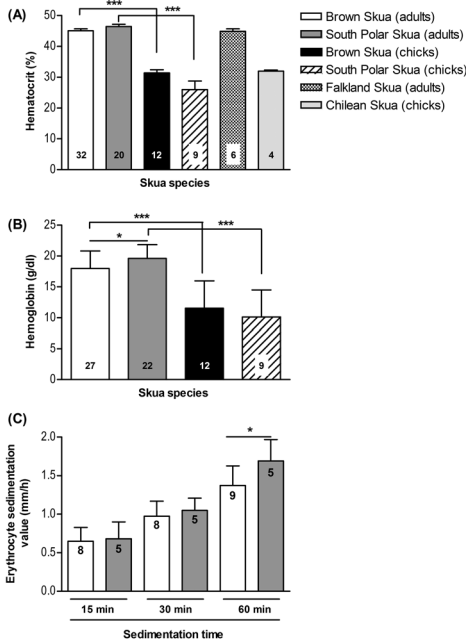


Figure 2. Hematological parameters of adult and chick Antarctic (Brown and South Polar) and non-Antarctic (Chilean and Falkland) skuas. (A) hematocrit (%), (B) hemoglobin concentration (g/dl), (C) erythrocyte sedimentation values (mm/h). Columns represent the mean and error bars of the SD; the sample size (*n*) is shown inside the columns; * = $P < 0.05$, *** = $P < 0.001$.

Falkland Skua vs. South Polar Skua: t -test $t_{24} = 1.056$, $P > 0.05$) or chicks (Chilean Skua vs. Brown Skua: t -test $t_{14} = 0.3019$, $P > 0.05$; Chilean Skua vs. South Polar Skua: t -test $t_{11} = 1.424$, $P > 0.05$) in this parameter (Fig. 2A).

Hemoglobin concentration in adult Antarctic skuas showed differences between species (t -test $t_{47} = 2.189$, $P < 0.05$), with values higher in South Polar Skuas. In addition, we found age-related differences in hemoglobin concentration in both species (Brown Skua: t -test $t_{37} = 5.44$, $P < 0.001$; South Polar Skua: t -test $t_{29} = 8.048$, $P < 0.001$) (Fig. 2B).

Erythrocyte sedimentation value was determined at different times (15, 30 and 60 min) in both Antarctic species. The South Polar Skua showed higher levels of this parameter at 60 min than the Brown Skua (t -test $t_{12} = 2.176$, $P = 0.05$), and at 15 and 30 min no differences were observed between species (15 min: t -test $t_{11} = 0.2716$, $P > 0.05$; 30 min: t -test $t_{11} = 0.7222$, $P > 0.05$) (Fig. 2C).

Biochemistry

Differences in total lipid concentration were observed between Antarctic skua species. Lipid concentration was higher in South Polar Skuas than in Brown Skuas (t -test $t_{19} = 3.935$, $P < 0.001$). On the other hand, there was no difference in lipid concentration among Brown Skua adults and chicks (t -test $t_{15} = 0.7513$, $P > 0.05$) (Fig. 3A).

The glucose concentration was similar in adult Antarctic skuas (t -test $t_{23} = 0.5119$, $P > 0.05$). However, glucose level in adults was higher than in chicks of each species (Brown Skua: t -test $t_{25} = 2.051$, $P = 0.05$; South Polar Skua: t -test $t_{11} = 3.227$, $P < 0.01$) (Fig. 3B).

Blood protein levels showed no differences between Antarctic skua species (t -test $t_{24} = 1.167$, $P > 0.05$). However, we found differences in protein concentration between Brown skua adults and chicks (t -test $t_{20} = 6.516$, $P < 0.001$), where mean values of chicks were 1.6 mg/dl higher than adults (Fig. 3C).

Blood concentration of AST and ALT was measured in adult Antarctic skuas. Concentration of AST was higher in Brown Skuas than in South Polar Skuas (t -test $t_{10} = 2.376$, $P < 0.05$) (Fig. 3D), while ALT levels were similar (t -test $t_{10} = 0.8180$, $P > 0.05$) (Fig. 3D). Finally, to determine the level of dehydration of sampled birds, in addition to hematocrit values, we measured electrolyte levels (sodium, potassium and chloride) in Brown Skuas (Table 1).

Protein Electrophoresis

The serum protein electrophoretic profile was analyzed for all species sampled in this study. Proteins were scattered in at least six peaks corresponding to prealbumin (pre-Alb), albumin (Alb), α -globulins (α_1 and α_2), β - and γ -globulin (Fig. 4). Baseline relative amounts for each fraction are described in Table 2. Differences among skua species were observed in α_1 , α_2 and β fractions, while the relative amount of albumin and γ -globulins was similar in all cases. In addition, similarities in the protein profile were found between South Polar and Chilean skuas and between Brown and Falkland skuas (Fig. 4).

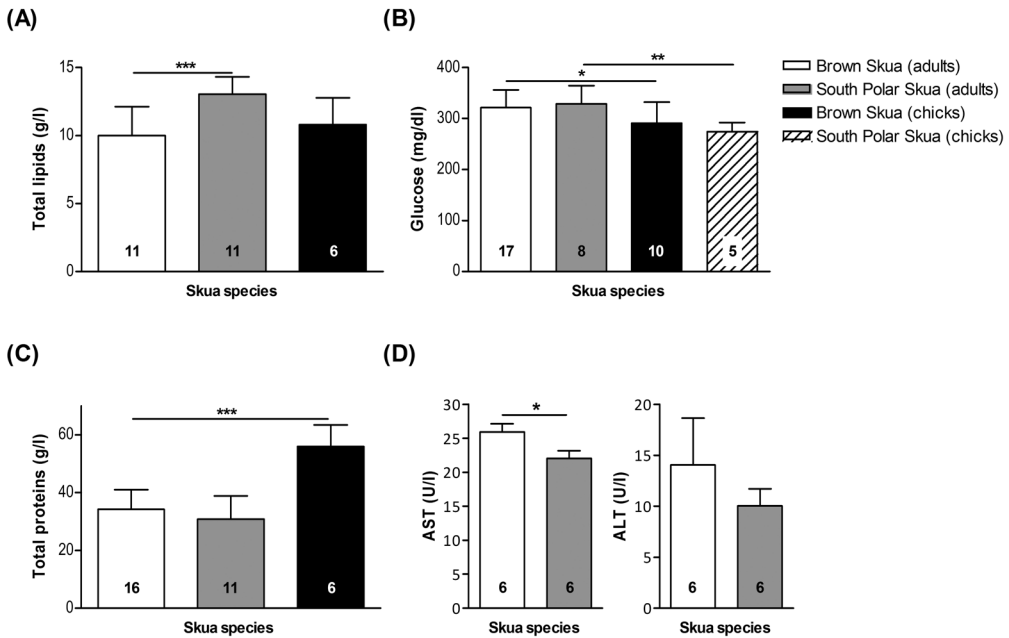


Figure 3. Biochemical parameters of Brown and South Polar skua adults and chicks. (A) total lipid concentration (g/l), (B) glucose concentration (mg/dl), (C) total proteins (g/l) and (D) aminotransferase AST and ALT activities (U/l). Columns represent mean and error bars of the SD; the sample size (n) is shown inside each column; * = $P < 0.05$, ** = $P < 0.005$ and *** = $P < 0.001$.

DISCUSSION

Hematological and biochemical values reported in this work for Antarctic skuas were similar to those previously described for these species (Milsom *et al.* 1973; Myrcha and Kostecka-Myrcha 1980; Rosa *et al.* 1993) and for other seabirds (Karesh *et al.* 1999; Uhart *et al.* 2003). Adult Antarctic skuas showed variations in hemoglobin, erythrocyte sedimentation, total lipids and aminotransferase activities. These variations may be attributed to differences in diet, feeding and migratory habits, and physiological adaptations during the breeding season (Newman *et al.* 1997). In addition, age-related differences were observed between

Antarctic skuas in hematocrit, hemoglobin, glucose and total protein concentration. These differences may be associated with physiological processes during development of chicks (Newman *et al.* 1997).

Adult South Polar Skuas showed higher hemoglobin concentration and erythrocyte sedimentation values than adult Brown Skuas, while similar hematocrit values were observed among adult Brown, South Polar and Falkland skuas. These differences between Antarctic skuas may be the result of an adaptation in oxygen affinity (Zhang *et al.* 2007) to perform different migratory flights. Previous reports described that South Polar Skuas migrate long distances during the Antarctic winter, performing a trans-equatorial

Table 1. Electrolyte concentration in Brown Skuas (n = 8). Data are mean (\pm SD) concentration (mEq/l) of sodium (Na^+), potassium (K^+) and chloride (Cl^-) and the minimum and maximum.

Electrolyte Concentration	Na^+ (mEq/l)	K^+ (mEq/l)	Cl^- (mEq/l)
Mean \pm SD	150.13 \pm 4.97	3.59 \pm 0.32	116.07 \pm 6.02
Minimum-Maximum	144.02-158.05	3.01-4.08	106.04-122.09

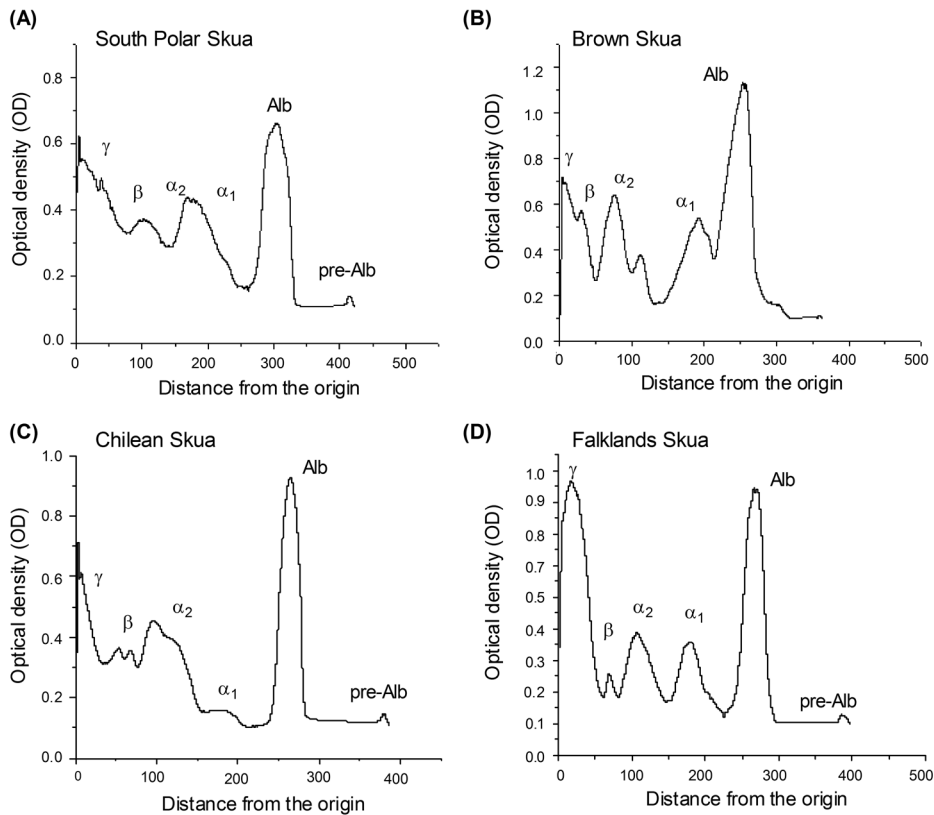


Figure 4. Serum electrophoretic profile of: (A) South Polar Skua, (B) Brown Skua, (C) Chilean Skua and (D) Falkland Skua. pre-Alb = prealbumin, Alb = albumin, α_1 and α_2 = alpha globulins, β = beta globulins and γ = gamma globulins.

migration to the north Atlantic and Pacific Oceans (Kopp *et al.* 2011), while the Brown Skuas reach the Atlantic Ocean and Pacific Ocean tropics (Phillips *et al.* 2007). Age-related differences in hematocrit and hemoglobin values between Antarctic skuas were detected (higher in adults) confirming the fact these parameters may vary during development and that oxygen requirement is higher in adults (Potti *et al.* 1999; Fair *et al.*

2007). Also, an increase in hematocrit is usually associated with dehydration (Campbell 1988). No dehydration was observed in adult Brown Skuas, since electrolyte levels were similar to those described for South Polar Skuas (Rosa *et al.* 1993).

Multiple blood metabolites (lipids, proteins and glucose) are regulated by dietary intake and metabolism and serve as an indicator of the nutritional status of wild

Table 2. Relative amount (%; mean \pm SD) of blood serum protein fractions for Antarctic (South Polar and Brown) and non-Antarctic (Chilean and Falkland) skuas.

Serum Protein Fraction (%)	South Polar Skua (n = 3)	Brown Skua (n = 3)	Chilean Skua (n = 2)	Falkland Skua (n = 2)
Albumin	26.43 \pm 2.70	36.22 \pm 1.86	29.61 \pm 0.92	28.91 \pm 1.28
α_1 -Globulins	28.92 \pm 1.04	20.36 \pm 1.37	8.99 \pm 0.76	17.13 \pm 1.24
α_2 -Globulins	17.03 \pm 0.37	7.71 \pm 2.21	30.01 \pm 3.42	17.81 \pm 3.52
β -Globulins	13.41 \pm 1.51	17.22 \pm 0.84	14.72 \pm 2.13	4.61 \pm 1.75
γ -Globulins	13.12 \pm 2.19	18.25 \pm 1.52	15.73 \pm 1.93	32.41 \pm 0.71

marine birds, which is strongly related to the health status. In this study, the differences in serum lipids between adult Antarctic skuas are likely related to feeding habits; Brown Skuas feed on terrestrial resources like eggs and penguin chicks (Graña Grilli and Montalti 2012), while South Polar Skuas primarily consume fishes with high fat content (Reinhardt and Van Vleet 1986; Reinhardt *et al.* 1997; Montalti *et al.* 2009). In Antarctic skuas, glucose values were similar between adults, while age-related differences (higher in adults) were observed. This last trend suggests a greater removal or supply of serum glucose in adult skuas that can be used as an energy source during breeding or feeding and migratory flights (Scanes and Braun 2013). Protein concentration can be influenced by the nutritional status, age, environment and inflammatory processes (Harr 2002). Serum protein concentration among adult Antarctic skuas was similar, but age variations in Brown Skuas (higher in chicks) were observed. Previous studies described higher protein levels in chicks (Dawson and Bortolotti 1997) as a consequence of their high absorption rate of food and the capacity of their livers to synthesize proteins. On the other hand, the lower levels in adults may be associated with stress and chronic inflammatory processes, or protein catabolism for energy supply during the breeding period when skuas were sampled (Newman *et al.* 1997; Harr 2002).

AST and ALT values are related to muscle damage, toxic indigestion and/or various metabolic disorders (Tlak *et al.* 2008). While ALT activity has limited clinical value in birds because it can be increased by pathological changes in almost all tissues, AST is considered to be a very sensitive indicator of hepatocellular disease and muscle damage (Harr 2002). The values of both enzymes in Antarctic skuas were similar to those described for other species (Tlak *et al.* 2008; García *et al.* 2010). In addition, the difference observed in AST between adult Antarctic skuas could be related to nutritional disorders or inflammatory processes that occurred during any breeding stage.

Protein electrophoresis is an invaluable diagnostic tool to assess avian physiological status by determining the relative or total amounts of albumin, α -, β -, and γ -globulin fractions (Grasman *et al.* 2000). Variation of these fractions is related to inflammatory processes, age and nutritional status. Moreover, by assessing the relative abundance and the protein fraction pattern, it is possible to evaluate inter-taxonomic relationships (Roman *et al.* 2009), as was described for Antarctic avian species (Murrish and McMahon 1975). The electrophoretic patterns described here represent useful and relevant data for future comparisons within species or between other species, and to infer ecological or nutritional constraints (Grasman *et al.* 2000; Najle *et al.* 2006). Also, the close correlation in the electrophoretic pattern and relative amount of serum protein in the South Polar and Chilean skuas suggests that these species are taxonomically related; the same type of close correlation was observed among Brown and Falkland skuas. These results support previous reports that identify close taxonomic relationships among these species, respectively (Furness 1996; Reinhardt *et al.* 1997).

Considering the multiple environmental factors that affect a seabird's body condition, the information reported here is relevant for establishing the reference physiological values and for comparison within the same species, or even with other species in different periods. The differences described in baseline blood parameters of Antarctic skuas, together with the existing works on breeding and diet, contribute to understanding the physiological and morphological adaptations that facilitate the use of different habitats and ecological niches in these sympatric species. However, no sex-specific determinations were performed in our study, and we acknowledge that differences between sexes can influence some biochemical parameters during the breeding season. Future sex-specific and long-term studies are necessary to establish seasonal baseline physiological values for a better understanding of the mechanisms responsible for variation in body condition that consequently affect reproductive

success in wild breeding populations. Once reference values are established for these species, clinical evaluations will improve and aid in future conservation efforts.

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