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BODY MASS AND PHYSIOLOGICAL VARIABLES OF INCUBATING MALES AND FEMALES IN THE EUROPEAN STORM PETREL (*HYDROBATES P. PELAGICUS*)

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ABSTRACT.—Examining body mass and physiological parameters in breeding adults may provide valuable insight into parental efforts provided by males and females at a given stage of breeding. Here, we examined body mass and physiological variables (hemoglobin and cholesterol concentrations, leukocyte profile) in males and females in a small pelagic seabird, the European Storm Petrel (*Hydrobates p. pelagicus*) during the incubation period. We expected females to be in poorer physiological condition compared to males because of their assumed higher investment (production of costly egg, with all other parental activities performed being similar). Contrary to our expectation, we did not find sex differences in the hematological values, indicating similar non-resource–based costs of reproduction. Also, we found females in good nutritional state, being heavier and having higher cholesterol concentration than males. Although we are not able to explicitly identify mechanisms responsible for the pattern observed, we suggest that common assumptions about female-biased efforts during the pre-laying and/or equal male and female parental efforts during incubation periods in the European Storm Petrel require verification. *Received 25 June 2015. Accepted 6 January 2016.*

Key words: body condition, *Procellariiformes*, reproductive investments, seabirds, sex differences.

Avian reproduction is considered to be costly as parents need to acquire extra time and energy to satisfy immediate demands of the raised offspring (Drent and Daan 1980). These efforts has been shown to lead to changes in body mass and various physiological variables of parenting individuals, accordingly to the amount of the efforts made (e.g., Taylor 1994, Moe et al. 2002, Williams et al. 2007). Examining body mass and physiological parameters in breeding adults may therefore offer insight into parental efforts provided by parents at a given stage of breeding. This may be particularly useful when examining bird species when their behavioral performance is logistically difficult to follow (e.g., pelagic species with night activity pattern).

In this study, we examined body mass and physiological variables in a small pelagic seabird, the European Storm Petrel (*Hydrobates p. pelagicus*) to assess male and female parental investment at the stage of the incubation period. Because of breeding in nest chambers hidden in the rocks, pelagic foraging at sea, and returning to the colony only at night, parental investment of males and females has never been studied in detail in this species, although both sexes are believed to share incubation and chick-feeding duties equally (Warham 1990, 1996), as other storm petrels *Hydrobatidae* do (e.g., Wilson's Storm Petrel *Oceanites oceanicus*; Gladbach et al. 2009). In general, males and females are anatomically, physiologically, and ecologically different; therefore, different physiological response of males and females for the same activities might be expected (Trivers 1972). At the initial stage of breeding, male and female parental performance is very much different, and the efforts are likely to be female-biased; females produce a large egg (20–30% of their body mass; Warham 1990, 1996; Sanz-Aguilar et al. 2012), and males guard their territories. Given all this, one may expect sex differences in physiological condition during the incubation period, when both males and females are assumed to do the same activities, but the initial female investment might not be yet restored.

To examine birds' physiological condition, we measured multiple variables including body mass, concentration of hemoglobin and cholesterol, and leukocyte profiles. Body mass, if appropriately corrected for body size, is a useful body condition estimate (Reist 1985, Jakob et al. 1996). In many seabirds, body mass decreases under conditions of food deprivation and in response to elevated efforts related to parental performance (e.g., Taylor 1994, Moe et al. 2002, Williams et al. 2007). Hemoglobin concentration is a measure of the oxygen-carrying capacity of blood (Campbell 1995). As such, it may serve as a relative measure of metabolic potential (Kostelecka-Myrcha 1997,

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Bańbura et al. 2007, Minias et al. 2013). There is some evidence that blood of more active species of birds, having higher metabolic requirements, has a higher hemoglobin concentration than that of less active species (e.g., Palomeque et al. 1980, Viscor et al. 1984). The concentration of cholesterol has been suggested to reliably indicate periods of food deprivation, as it decreases with reduction of body mass (Alonso-Alvarez et al. 2002). The leukocyte profiles provide a convenient measure of integrated immune function (Davis 2005, Salvante 2006, Davis et al. 2008), with lymphocytes and heterophils constituting majority of leucocytes and being responsible for pathogen-specific (lymphocytes), and non-specific (heterophils) immune response (Campbell 1995). In consequence, both lymphocytes and heterophils increase during immunological challenges (Eeva et al. 2005). The heterophil to lymphocyte ratio (H/L) is often used as a stress indicator in birds (Maxwell 1993, Davis et al. 2008). It is known to increase along with efforts associated with reproduction (mostly through a decrease in number of lymphocytes), when birds facing the problem of limited energy resources reach a trade-off by partially suppressing a part of their immune system (Salvante 2006, Davis et al. 2008).

Assuming female-biased efforts at the initial stage of breeding, and equal sharing of incubation duty, we expect that females are in worse physiological condition compared to males. Consequently, we expect that females have lower body mass and cholesterol concentration, and elevated hemoglobin concentration and H/L ratios compared to males. Additionally, by measuring several variables simultaneously, we investigate their inter-relationships to get insight into the potential mutual influence of the studied variables.

MATERIALS AND METHODS

We carried out the study during 4 consecutive days from 6–9 August 2013 in a large breeding colony of the European Storm Petrel located on Nólsoy Island (61°59′N, 06°47′W) in the Faeroe Archipelago. We captured 67 breeders with well-developed brood patches by using mist-nets. Based on monitoring of a few accessible nests, and birds' behavior in the colony, we made an assumption that we captured the birds in the second week of the incubation period. Since timing of breeding in social seabirds is usually quite synchronized (Hamer et al. 2001), and that is particularly the case for egg-laying (Henson et al. 2010), we believe that the birds we captured were in a very similar phase of the incubation period. We marked all birds with a metal ring (Natural History Museum of Denmark), aged them following Bolton and Thomas (2001), and checked breeding status (individuals with brood patch present were considered to be breeders).

From each breeder, we took several measurements using a ruler with 1-mm accuracy (wing length) and caliper with 0.1-mm accuracy (length of tail, bill and head-bill, bill depth and bill width, see Jakubas et al. [2014] for details) to find the best variable describing bird body size. We weighed all birds using an electronic balance with 0.1-g accuracy and took a blood sample (~20 μL) from individuals by puncturing a brachial vein with a sterile, disposable needle (Owen 2011). Immediately after sampling, we divided the blood taken into four subsamples in order to: a) analyze the concentration of hemoglobin (5 μL) using a portable hemoglobin photometer (Hb 201 System; HemoCue, Ängelholm, Sweden); (b) analyze the concentration of cholesterol (5 μl) using standard, commercial strips and a portable photometer (CardioChek 1709; PTS Diagnostics, Indianapolis, IN, USA); (c) make an air-dried blood smear for examining the leukocyte profile (~5 μL); and (d) preserve material in 96% pure ethanol for molecular sexing (the remaining blood volume). For some individuals, the amount of blood taken was not sufficient to perform the whole set of analyses; therefore, the sample size given varied across the studied variables. In 11% of samples, the values of cholesterol concentrations were below the range specified by the CardioChek producer. In such cases, we assigned the lowest possible value detected by the photometer used (i.e., 2.59 mmol/l) to the sampled individual. We released all birds unharmed after ~10 min of handling.

We stained the blood smears with the May-Grünewald-Giemsa method (Lillie 1977) with Wescor 'Aerospray Hematology' stainer ~3 weeks after blood sampling. To determine the leukocyte profiles, we examined one-cell layer, non-overlapping microscope fields of each smear at 1000 × magnification under oil immersion. This was

performed up to achieving 100 leukocytes, regardless of their type (on average 79 fields examined). The relative numbers of each type of leukocytes were calculated as the percentage of all leukocytes. Additionally, we estimated the total number of leukocytes and calculated abundance of the most common types of the white blood cells (heterophils, lymphocytes) per 10,000 RBC (red blood cells). This was done by counting red blood cells per field and multiplying the outcome by the number of fields within which leukocytes had to be counted to reach 100, following Lobato et al. (2005) who found high repeatability of this method. All leukocyte counts were performed by the same person (AK), oblivious of the status of the sampled bird (sex, other hematological variables).

We extracted DNA from blood following evaporation of the alcohol using the Blood Mini kit (A&A Biotechnology, Gdynia, Poland). We performed molecular sexing with primer pair 2550F and 2718R according to the protocol described by Griffiths et al. (1998), using 50°C for the annealing temperature in the polymerase chain reaction. The primers amplify introns on the CHD-W and CDH-Z genes located on the W and Z avian sex chromosomes that vary in length (Griffiths et al. 1998). The difference between the two fragments (~200 bp) was clearly visible in UV-light when separating on 2% agarose gel, stained with Midori Green. We sexed all birds successfully. Thus, in total, we present results for 40 males and 27 females.

To correct body mass for body size, we used scaled mass index (SMI), recently proposed by Peig and Green (2009). It standardizes all individuals to the same body size, adjusting their body mass to the one they would have at their new body size in accordance with the scaling trend between body mass and body size (Peig and Green 2009, 2010). To calculate the index, we used following formula (Peig and Green 2009):

$$\text{Scaled mass index (SMI)} = M_i \left[\frac{L_o}{L_i}\right]^{b_{SMA}},$$

where M_i and L_i are the mass and linear body measurement (overall head-bill length) of individual *i,* respectively, and b_{SMA} is the scaling exponent estimated from the regression of M and L. L_o is the arithmetic mean value of the linear measurement. We used mean value of the overall head-bill length for the studied population. We choose this variable, because correlation of this measurement with the body mass was significant, with the highest correlation and determination coefficients among nine body measurements studied ($r = 0.33$, $R^2 = 0.11$, $t_{65} = 2.86$, $P = 0.006$). The scaling exponent was calculated by dividing the slope of the ordinary linear square regression of $\ln M$ and $\ln L$ by the Pearson's correlation coefficient (LaBarbera 1989, Peig and Green 2009).

Before comparing the studied variables between the sexes, we tested their normality (Shapiro-Wilk test), and homogeneity of variance (Levene's test). If the variable did not meet the assumptions, we normalized it using an arcsin square-root transformation (Zar 1999). This was the case for H/L, and the number of the heterophils and lymphocytes per 10,000 erythrocytes. An apparent outlier, a value beyond 2 SD for cholesterol concentration in males, was excluded before further analyses. Because of very low numbers or absence of basophils, eosinophils, and monocytes, we did not consider these leukocytes in statistical analyses and presented only their abundance per 100 leukocytes as reference values (means, SD, min–max range) in Table 1.

We compared all variables between sexes using Student *t*-tests for independent variables. To analyze the relationships between assorted variables, we used Pearson's correlation. Following Moran's (2003) recommendation, since we correlated only variables that we assumed to be biologically related to each other, there was no use to apply a multiple comparisons correction. We performed all calculations and analyses in R (version 3.1.2; R Core Team 2014).

RESULTS

We found significant sex differences in the scaled mass index, with females being heavier than males (Table 1). We also recorded significantly higher concentration of cholesterol in females compared to males (Table 1). We did not find sex differences in any other hematological variables, i.e. hemoglobin concentration, the H/L ratio, and number (per 10,000 erythrocytes) of heterophils,

TABLE 1. Reference values for body mass and physiological variables in male and female European Storm Petrels during the incubation period, with test for intersexual difference (Student *t*-tests, significant differences [$P < 0.05$] in bold).

Studied variables	Males Mean ± SD (min–max), *n*	Females Mean ± SD (min–max), *n*	*t*	*P*
Scaled body mass index	24.61 ± 3.32 (19.43–33.4), 40	26.48 ± 2.62 (22.1–32.9), 27	2.34	**0.02**
Hemoglobin concentration [g L^{-1}]	169.00 ± 16.24 (112–192), 39	172.70 ± 10.70 (144–191), 27	0.91	0.37
Cholesterol concentration [mmol L^{-1}]	4.15 ± 1.26 (2.59–7.17), 37	5.00 ± 1.55 (2.59–7.71), 27	2.34	**0.02**
Heterophils/lymphocytes ratio	1.23 ± 0.56 (0.22–3.00), 39	1.19 ± 0.73 (0.14–2.85), 25	−0.87	0.39
N heterophils per 10,000 RBC	68.67 ± 20.85 (33.0–139.3), 39	63.78 ± 19.43 (24.1–94.5), 25	−0.95	0.35
N lymphocytes per 10,000 RBC	62.74 ± 25.46 (34.1–188.9), 39	72.48 ± 44.57 (32.5–206.1), 25	0.86	0.40
Total N leukocytes per 10,000 RBC	136.80 ± 28.76 (88.5–234.9), 39	141.70 ± 36.14 (103.7–245.3), 25	0.58	0.57
Relative number of basophiles [%]	0.25 ± 0.53 (0–2), 39	0.19 ± 0.40 (0–1), 25	–	–
Relative number of eosinophils [%]	0.83 ± 1.16 (0–5), 39	0.87 ± 1.03 (0–3), 25	–	–
Relative number of monocytes [%]	0.76 ± 0.93 (0–3), 39	0.89 ± 1.06 (0–4), 25	–	–

lymphocytes, and total number of leukocytes (Table 1).

By analyzing relationships between the variables in males, we found only one significant correlation: hemoglobin concentration decreased with increasing number of heterophils (Table 2). We found the same negative relationship in females (Table 2). Moreover, in females we found positive significant relationships between cholesterol concentration and three leukocyte variables (the H/L ratio, number of lymphocytes, and total leukocytes; Table 2).

TABLE 2. Relationships between particular body and physiological condition variables in male European Storm Petrels (above diagonal), and females (below diagonal) during the incubation period (Pearson correlation). Significant ($P < 0.05$) relationships in bold. NA = not analyzed.

	Males						
Females	Scaled body mass index	Hemoglobin concentration	Cholesterol concentration	Heterophils/ lymphocytes ratio	N heterophils per 10,000 RBC	N lymphocytes per 10,000 RBC	Total n leukocytes per 10,000 RBC
Scaled body mass index	–	$r = -0.13$ $t_{37} = -0.78$ $P = 0.44$	$r = -0.26$ $t_{36} = -1.62$ $P = 0.11$	$r = 0.25$ $t_{37} = 1.58$ $P = 0.12$	$r = 0.24$ $t_{37} = 1.53$ $P = 0.13$	$r = -0.11$ $t_{37} = -0.69$ $P = 0.49$	$r = 0.11$ $t_{37} = 0.68$ $P = 0.50$
Hemoglobin concentration	$r = 0.18$ $t_{25} = 0.92$ $P = 0.36$	–	$r = -0.03$ $t_{35} = -0.15$ $P = 0.88$	$r = -0.21$ $t_{36} = -1.32$ $P = 0.19$	$\mathbf{r = -0.35}$ $\mathbf{t_{36} = -2.22}$ $\mathbf{P = 0.03}$	$r = 0.04$ $t_{36} = 0.211$ $P = 0.83$	$r = -0.18$ $t_{36} = -1.11$ $P = 0.27$
Cholesterol concentration	$r = -0.13$ $t_{25} = -0.67$ $P = 0.50$	$\mathbf{r = -0.43}$ $\mathbf{t_{25} = -2.40}$ $\mathbf{P = 0.02}$	–	$r = 0.15$ $t_{36} = 0.89$ $P = 0.38$	$r = 0.05$ $t_{36} = 0.30$ $P = 0.76$	$r = -0.20$ $t_{36} = -1.22$ $P = 0.23$	$r = -0.18$ $t_{36} = -1.11$ $P = 0.27$
Heterophils/ lymphocytes ratio	$r = 0.30$ $t_{23} = 1.48$ $P = 0.15$	$r = -0.08$ $t_{23} = -0.36$ $P = 0.72$	$\mathbf{r = 0.44}$ $\mathbf{t_{23} = 2.34}$ $\mathbf{P = 0.02}$	–	NA	NA	NA
N heterophils per 10,000 RBC	$r = 0.35$ $t_{23} = 1.78$ $P = 0.87$	$r = 0.04$ $t_{23} = 0.20$ $P = 0.84$	$r = 0.35$ $t_{23} = 1.79$ $P = 0.08$	NA	–	NA	NA
N lymphocytes per 10,000 RBC	$r = -0.12$ $t_{23} = -0.57$ $P = 0.57$	$r = 0.18$ $t_{23} = 0.89$ $P = 0.38$	$\mathbf{r = -0.47}$ $\mathbf{t_{23} = -2.52}$ $\mathbf{P = 0.01}$	NA	NA	–	NA
Total N leukocytes per 10,000 RBC	$r = 0.09$ $t_{23} = 0.43$ $P = 0.67$	$r = 0.25$ $t_{23} = 1.24$ $P = 0.22$	$r = -0.37$ $t_{23} = -1.91$ $P = 0.07$	NA	NA	NA	NA

DISCUSSION

We expected European Storm Petrel females that were incubating to be in worse physiological condition compared to males because of their earlier investment into the costly egg. This initial investment might not have been compensated at the stage of incubation, if the two sexes are supposed to share the incubation duty equally. Contrary to our expectation, we did not find sex differences in the hematological values. Also, we found females in good nutritional state as they were heavier and had higher cholesterol concentrations than males.

There are two, non-exclusive explanations of the unexpected direction of the sex differences in physiological condition. Firstly, the assumption of female-biased parental investment made up so far might not be correct. There are multiple possible scenarios of behavioral performance to achieve given sex differences in the physiological condition. For example, females could compensate cost of egg production during early incubation period. Although both sexes might share incubation duties (Warham 1990, 1996), females could spend more time out of the colony than males, which could positively affect their nutritional state. Such a pattern of sex differences in physiological condition and parental performance during the incubation period has been found to be the case in another small seabird, the Little Auk *Alle alle* (Jakubas et al. 2008, Wojczulanis-Jakubas et al. 2009). Apparently, the results presented here highlight the need for behavioral studies of the European Storm Petrel, to verify common assumptions about female-biased parental investment at the initial stage of breeding and/or equal male and female efforts at the incubation stage.

Secondly, faced with energetically costly egg production, females may have foraged extensively during the egg production and/or just after egg laying, and so gained body mass and obtained relatively high cholesterol concentration. Such interpretation seems to be supported by the significant relationships between cholesterol concentration and the H/L ratio (positive) and number of lymphocytes per 10,000 RBC (negative) found exclusively in females. The H/L ratio increases, mostly through a decrease in the number of lymphocytes per 10,000 RBC, when birds face the problem of limited energy resources (Salvante 2006, Davis et al. 2008). Thus, elevated H/L ratio and lowered number of lymphocytes in females suggest that they indeed might have faced an energetically challenging situation related to the egg production. For that reason, they could have temporarily switched-off the costly acquired immunity (represented by lymphocytes; Lochmiller and Deerenberg 2000, Norris and Evans 2000). Simultaneously, higher body mass and cholesterol concentration in females suggest that they foraged extensively, which is likely to be related to the egg production.

In the present study, we evaluated multiple variables, most of which have been examined in the European Storm Petrel for the first time. With the note that the birds were examined in a single site and season, the values presented may serve as reference ones for the incubation period. In many studies, birds' physiological condition is examined using a single variable (e.g., only body mass corrected for size). The results presented here, showing significant sex differences in some variables but not in others, along with mutual relationships between the variables, indicate that the single variable approach may be misleading. Therefore, whenever physiological condition of birds is examined, at least two parameters of different nature should be monitored to interpret results appropriately.

Higher body mass corrected for size and cholesterol concentration in female European Storm Petrels compared to males suggest some sex differences in the form of parental investment performed up to the incubation period. Although we were not able to indicate mechanisms responsible for the pattern observed, we showed that common assumptions about female-biased efforts during pre-laying stage and/or equal male and female parental efforts during the incubation period in the European Storm Petrel require verification.

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LITERATURE CITED

Alonso-Alvarez, C., M., Ferrer, and A. Velando. 2002. The plasmatic index of body condition in Yellow-legged Gulls *Larus cachinnans*: a food-controlled experiment. Ibis 144:147–149.

Bańbura, J., M. Bańbura, A. Kaliński, J. Skwarska, R. Słomczyński, J. Wawrzyniak, and P. Zieliński. 2007. Habitat and year-to-year variation in haemoglobin concentration in nestling Blue Tits *Cyanistes caeruleus*. Comparative Biochemistry and Physiology, Part A 148:572–577.

Bolton, M. and R. Thomas. 2001. Moult and ageing of storm petrels *Hydrobates pelagicus*. Ringing and Migration 20:193–201.

Campbell, T. W. 1995. Avian hematology and cytology. Second Edition. Iowa State University Press, Ames, USA.

Davis, A. K. 2005. Effect of handling time and repeated sampling on avian white blood cell counts. Journal of Field Ornithology 76:334–338.

Davis, A. K., D. L. Maney, and J. C. Maerz. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology 22:760–772.

Drent, R. H. and S. Daan. 1980. The prudent parent: energetic adjustments in avian breeding. Ardea 68:225–252.

Eeva, T., D. Hasselquist, Å. Langefors, L. Tummeleht, M. Nikinmaa, and P. Ilmonen. 2005. Pollution related effects on immune function and stress in a free-living population of Pied Flycatcher *Ficedula hypoleuca*. Journal of Avian Biology 36:405–412.

Gladbach, A., C. Braun, A. Nordt, H.-U. Peter, and P. Quillfeldt. 2009. Chick provisioning and nest attendance of male and female Wilson's Storm Petrels *Oceanites oceanicus*. Polar Biology 32:1315–1321.

Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7:1071–1075.

Hamer, K. C., E. A. Schreiber, and J. Burger. 2001. Breeding biology, life histories, and life history–environment interactions in seabirds. Pages 217–262 *in* Biology of marine birds (E. A. Schreiber and J. Burger, Editors). CRC Press. Boca Raton, Florida, USA.

Henson, S. M, J. L. Hayward, J. M. Cushing, and J. G. Galusha. 2010. Socially induced synchronization of every-other-day egg laying in a seabird colony. Auk 127:571–580.

Jakob, E. M., S. D. Marshall, and G. W. Uetz. 1996. Estimating fitness: a comparison of body condition indices. Oikos 77:61–67.

Jakubas, D., K. Wojczulanis-Jakubas, and J.-K. Jensen. 2014. Body size variation of European Storm Petrels *Hydrobates pelagicus* in relation to environmental variables. Acta Ornithologica 49:71–82.

Jakubas, D., K. Wojczulanis-Jakubs, and R. Kreft. 2008. Sex differences in body condition and hematological parameters in Little Auk *Alle alle* during the incubation period. Ornis Fennica 85:90–97.

Kostelecka-Myrcha, A. 1997. The ratio of amount of haemoglobin to total surface area of erythrocytes in birds in relation to body mass, age of nestlings, and season of the year. Physiological Zoology 70:278–282.

LaBarbera, M. 1989. Analyzing body size as a factor in ecology and evolution. Annual Review of Ecology, Evolution, and Systematics 20:97–117.

Lillie, R. D. (Editor). 1977. H. J. Conn's biological stains: a handbook on the nature and uses of the dyes employed in the biological laboratory. Ninth Edition. Williams and Wilkins Co., Baltimore, Maryland, USA.

Lobato, E., J. Moreno, S. Merino, J. J. Sanz, and E. Arriero. 2005. Haematological variables are good predictors of recruitment in nestling Pied Flycatchers (*Ficedula hypoleuca*). Ecoscience 12:27–34.

Lochmiller, R. L. and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88:87–98.

Maxwell, M. H. 1993. Avian blood leukocyte responses to stress. World's Poultry Science Journal 49:34–43.

Minias, P., K. Kaczmarek, R. Włodarczyk, and T. Janiszewski. 2013. Low oxygen-carrying capacity of blood may increase developmental instability of molt in migrating waders. Auk 130:308–312.

Moe, B., I. Langseth, M. Fyhn, G. W. Gabrielsen, and C. Bech. 2002. Changes in body condition in breeding kittiwakes *Rissa tridactyla*. Journal of Avian Biology 33:225–234.

Moran, M. D. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100:403–405.

Norris, K. and M. R. Evans. 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behavioral Ecology 11:19–26.

Owen, J. C. 2011. Collecting, processing, and storing avian blood: a review. Journal of Field Ornithology 82:339–354.

Palomeque, J., J. D. Rodriguez, L. Palacios, and J. Planas. 1980. Blood respiratory properties of swifts. Comparative Biochemistry and Physiology, Part A 67:91–95.

Peig, J. and A. J. Green. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118:1883–1891.

Peig, J. and A. J. Green. 2010. The paradigm of body condition: a critical reappraisal of current methods based on mass and length. Functional Ecology 24:1323–1332.

R Core Team. 2014. R: a language and environment for statistical computing. Version 3.1.2. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org

Reist, J. D. 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Canadian Journal of Zoology 63:1429–1439.

Salvante, K. G. 2006. Techniques for studying integrated immune function in birds. Auk 123:575–586.

Sanz-Aguilar, A., E. Mínguez, and D. Oro. 2012. Is laying a large egg expensive? Female-biased cost of first reproduction in a petrel. Auk 129:510–516.

Taylor, J. R. E. 1994. Changes in body mass and body reserves of breeding Little Auks (*Alle alle* L.). Polish Polar Research 15:147–168.

Trivers, R. L. 1972. Parental investment and sexual selection. Pages 136–179 *in* Sexual selection and the descent of man, 1871–1971 (B. Campbell, Editor). Aldine, Chicago, Illinois, USA.

Viscor, G., J. Fuentes, and J. Palomeque. 1984. Blood rheology in the pigeon (*Columba livia*), hen (*Gallus gallus domesticus*), and Black-headed Gull (*Larus ridibundus*). Canadian Journal of Zoology 62:2150–2156.

Warham, J. 1990. The petrels: their ecology and breeding systems. Academic Press, London, United Kingdom.

Warham, J. 1996. The behaviour, population biology and physiology of the petrels. Academic Press Ltd., London, United Kingdom.

Williams, C. T., S. D. Kildaw, and C. L. Buck. 2007. Sex-specific differences in body condition indices and seasonal mass loss in Tufted Puffins. Journal of Field Ornithology 78:369–378.

Wojczulanis-Jakubas, K., D. Jakubas, and L. Stempniewicz. 2009. Sex-specific parental care by incubating Little Auks (*Alle alle*). Ornis Fennica 86:140–148.

Zar, J. H. 1999. Biostatistical analysis. Fourth Edition. Prentice Hall, Upper Saddle River, New Jersey, USA.