Plasma chemistry and hematology reference values in wild nestlings of White-tailed Sea Eagles (Haliaeetus albicilla): effects of age, sex and hatching date

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ORIGINAL ARTICLE

Plasma chemistry and hematology reference values in wild nestlings of White-tailed Sea Eagles (Haliaeetus albicilla): effects of age, sex and hatching date

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Abstract

Studies on normal concentration of blood constituents of free-living birds are not very common. An adequate knowledge of blood chemistry is greatly recommended for those projects involving research and management of populations as far as they can be valuable for the assessment of the nutritional levels and health status of species. No previous published reports on these parameters from this species are available. The aim of this study was to obtain representative reference measures for hematologic and biochemical values in free-living clinically healthy wild White-tailed Sea Eagle nestlings (*Haliaeetus albicilla*). In addition, we investigated potential relationships between blood parameters, sex, age and hatching dates. Blood samples were obtained as part of routine monitoring and management when wild chicks were removed from their nest as part of a reintroduction program prerelease health check. A total of 83 nestlings, 43 males and 40 females, between 41 and 66 days of age (mean = 54.22, SD = 5.7), were sampled. Significant differences between sexes were found. Among hematological parameters, MCH, lymphocytes P and thrombocytes showed significant differences between males and females. In biochemical parameters, significant differences were found only in calcium, CK and LDH between sexes. No effect was found of age of the nestling when the sample was taken in any of the analyzed hematological parameters. No other significant relationships were found between biochemical parameters and other considered explanatory variables. Hatching date showed no relationship with blood parameters excepting urea. Urea was the only variable showing a strong relationship with hatching date, with those nestlings hatching later in the season showing higher urea concentration.

Keywords Blood parameters · Hatching date · Plasma chemistry · Hematology · Urea · Cholesterol · Free-living raptor

Zusammenfassung

Plasmachemie und hämatologische Referenzwerte bei wildlebenden Seeadlernestlingen *Haliaeetus albicilla*: Auswirkungen von Alter, Geschlecht und Schlupfdatum

Es gibt nicht sehr viele Untersuchungen zur Normalkonzentration von Blutbestandteilen bei wildlebenden Vögeln. Für Projekte, welche Populationsstudien und -management beinhalten, ist eine hinreichende Kenntnis der Blutchemie ratsam,

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da diese hilfreich bei der Beurteilung des Ernährungs-und Gesundheitszustands der Arten sein kann. Bisher gibt es in der Literatur noch keine Arbeiten über diese Parameter bei dieser Art. Ziel dieser Studie war es, repräsentative Referenzbereiche für hämatologische und biochemische Werte klinisch gesunder, wildlebender Seeadlernestlinge *Haliaeetus albicilla* zu erhalten. Zusätzlich untersuchten wir potenzielle Beziehungen zwischen Blutparametern, Geschlecht, Alter und den Schlupfdaten. Die Blutproben wurden im Rahmen routinemäßiger Monitoring- und Managementmaßnahmen gewonnen, wenn die wildlebenden Küken für einen zum Wiederansiedlungsprogramm gehörigen Gesundheitsscheck vor der Freilassung dem Nest entnommen wurden. Insgesamt beprobten wir 83 Nestlinge, darunter 43 Männchen und 40 Weibchen, im Alter zwischen 41-66 Tagen (Mittelwert = 54,22, Standardabweichung (SD) = 5,7). Wir entdeckten signifikante Unterschiede zwischen den Geschlechtern. Bei den hämatologischen Parametern zeigten sich bei MCH, P-Lymphozyten und Thrombozyten signifikante Unterschiede zwischen Männchen und Weibchen. Bei den biochemischen Parametern gab es nur bei Kalzium, CK und LDH signifikante Unterschiede. Wir konnten keinen Einfluss des Nestlingsalters bei der Probennahme auf einen der untersuchten hämatologischen Parameter feststellen. Bei den biochemischen Parametern verhielt es sich genauso. Mit Ausnahme von Harnstoff stand der Schlupftermin in keiner Beziehung zu den Blutparametern. Harnstoff stand als einzige Variable in einem engen Zusammenhang mit dem Schlupfdatum, insofern als später in der Saison geschlüpfte Nestlinge eine höhere Harnstoffkonzentration aufwiesen.

Introduction

Avian blood chemistry is used in ornithological studies because it provides biological data and allows the detection of possible pathological states (Meredith et al. 2012). Determination of physiological conditions can be very important in the understanding of ecological and behavioral issues (Ferrer et al. 2013; Ferrer and Morandini 2017; Ferrer et al. 2017a, b; Morandini et al. 2018; Morandini and Ferrer 2019). Hematological studies carried out on several aspects of the biochemistry and physiology of birds have increased, in particular, those concerned with the determination of normal values of blood chemistry parameters (Gee et al. 1981; Polo et al. 1992; Ferrer and Dobado-Berrios 1998; Ibañez et al. 2015; Gomez-Ramirez et al. 2016; Seok et al. 2017; Doussang et al. 2018).

However, studies on normal concentration of blood constituents of free-living birds are not very common, and less than 5% of the species of birds have been studied, mostly in captivity (for example, Balasch et al. 1976; Gee et al. 1981; Ferrer et al. 1987; Lumeij and Remple 1991; Polo et al. 1992; Garcia-Montijano 2002; Doussang et al. 2018). An adequate knowledge of blood chemistry is greatly recommended for those projects involving research and management of populations as far as they can be valuable for the assessment of the nutritional levels and health status of species (Ferrer and Dobado-Berrios 1998; Meredith et al. 2012; Ferrer et al. 2017a, b). Additionally, many hypotheses in behavioral ecology rely in differences among individuals in nutritional levels (Ferrer 1992a, b; Ferrer 1993; Morandini and Ferrer 2019). These studies carried on in long-lived birds are important providing basic data that are interesting in veterinary medicine, taxonomy and ecology (Balasch et al. 1976; Polo et al. 1992; Aguilera et al. 1993; Meredith et al. 2012; Ferrer et al. 2017a; Morandini et al. 2018).

Hematological variables, including chemical components, are known to be influenced by many factors: physiological state, age, sex, nutritional condition, circadian rhythm, seasonal changes, captivity, pollutants and plasma storing methods (Gee et al. 1981; Rehder and Bird 1983; Ferrer et al. 1987; Garcia-Rodriguez et al. 1987a, b; Viñuela et al. 1991; Jenni-Eiermann and Jenni 1992). When studying the influence of any of these factors, researchers must be sure that the others are controlled (Ferrer 1990). Most studies are focus mainly on publishing reference intervals or questions of clinical interest—both very important areas of research. However, the data gathered in analyzing plasma biochemistry can provide insight into the physiological state of an individual with implications for ecology (Gee et al. 1981; Ferrer 1992a, b).

The aim of this study was to obtain representative reference measures for hematologic and biochemical values in free-living clinically healthy wild white-tailed sea eagle nestlings (*Haliaeetus albicilla*). In addition, we investigated potential relationships between blood parameters, sex, age and hatching dates.

Materials and methods

Study area and species

White-tailed sea eagle (WTSE) is a large bird of prey with females being larger than males. The species is Eurasian in distribution and ecologically the Old World equivalent of the New World's Bald Eagle (*Haliaeetus leucocephalus*). Our study was involved with the second phase (east Scotland) of a reintroduction initiative instigated by an initial (1975–1985: 82 birds) reintroduction and later reinforcement translocations (1993–1995: 59 birds) using birds from western Norway to the west coast of Scotland (Green et al. 1996; Evans et al. 2009; Whitfield

et al. 2009a, b; Sansom et al. 2016). The core initiative allowing our study aimed to provide a further nucleus of WTSE population growth on the east coast of Scotland, situated away from the previous centers of population re-establishment and subsequent expansions on the west coast.

We sampled nestlings extracted from a wild donor WTE population in Norway: every June between 2007 and 2012 WTE nestlings were collected from monitored nests in the western counties of Møre og Romsdal, Hordaland and Sogn og Fjordane. Single birds were extracted from nests that had two or three chicks which from field observations were deemed old enough (five to eight weeks old) to be suitable for translocation on plumage features and physical appearance (Helander 1981). Extractions were coordinated with the goal of a balanced sex ratio (based on biometrics). Birds were uniquely metal banded (BTO: British Trust for Ornithology) in Norway prior to translocation, for subsequent identification.

There were extractions of 15 (2007, 2008, 2009), 19 (2010), 16 (2011) and six (2012) nestlings resulting in 86 translocated birds. (Eighty-five were released as one 2009 bird died in captivity from aspergillosis, despite veterinary intervention.) After transport from Norway, all birds had a full clinical checkup within 24 h of arrival to identify any potential clinical or pathological abnormalities. Translocated birds were kept at a hacking facility in northeast Fife, southeast Scotland, in wooden (three sides enclosed) and wire mesh aviaries measuring approximately $3.6 \times 3 \times 2.7$ m that had "nesting" platforms covered in bark chips and soft vegetation, and at least two long perches. Two or three birds were housed in each aviary, with similarly sized birds being kept together. Birds were fed ad libitum with the wide range exploited by WTSE such as fish, birds and mammals, with vitamin and mineral supplements (Nutrobal®, Vertak, UK) added to the food daily. Contact with humans was minimized by food being provided via a hatch in the aviaries' back panel. Captive eagles were kept under daily observation via "peep holes" in aviaries' back panels for surveillance of their status and any signs of injury or ill-health, with a qualified veterinarian on call should the need arise. No eagles were injured because of our activities.

The sampling was undertaken shortly after all birds were in the hacking facility. This allowed for greater efficiency in sampling all birds at the same time after extraction and transportation from Norway to Scotland. Birds were aged (days since hatch date) using the method of Løseth et al. (2019): after T. Nygård pers. comm.) involving measurement of a central tail feather. This method was preferred to those in Helander (1981) and Helander et al. (2007) involving other recorded biometrics when like Løseth et al. (2019), we also found the "Nygård" method to produce more realistic and consistent results.

Blood collecting procedures, body measurements and sex determination

The blood samples were drawn from nestlings 41–66 d (mean = 52.4, SD = 5.7) after their estimated hatch, corresponding to the beginning of hacking. Blood samples were collected between 11:00 AM and 15:00 PM to avoid variations in blood parameters due to circadian rhythms (Garcia-Rodriguez et al. 1987b; Ferrer 1990; Ferrer et al. 1993).

Blood samples were collected from the cutaneous ulnar (brachial) vein with the birds cast in dorsal recumbency. Blood was collected in a 10-ml plastic syringe (Becton Dickinson S.A., Spain) attached to a 23 gauge 1″ needle (Monoject, USA). Three blood smears were made using a slide-on-slide technique. One ml of blood was transferred to a di-potassium ethylene diamine tetra acetate (EDTA) anticoagulant tube (Teklab UK) for "DNA" sexing. Birds were subsequently sexed at a ("DNA") molecular level according to methods described by Ogden et al. (2015). An additional 1-ml EDTA tube was filled and submitted for hematological analysis. A 1-ml tube containing lithium heparin (Teklab, UK) as an anticoagulant was filled for blood lead analysis. The remaining sample was transferred to a 6-ml lithium heparin tube (Becton Dickinson S.A., Spain) and submitted for biochemical analyses. All samples were kept in a cooler box at approximately 4 °C for a maximum of 8 h prior to submission for laboratory analysis. Samples arrived within 24 h at Greendale Veterinary Diagnostics, Surrey, UK, where biochemical and hematologic analyses were performed. Blood lead analysis was performed at Veterinary Laboratory Agencies (VLA) UK. Packed cell volumes and total plasma protein levels were also examined within three hours of collection at the Veterinary Centre, University of Edinburgh, UK.

Hematologic analyses

Red blood cell (RBC) counts were measured using an impedance counter with a floating threshold by a CellTac analyzer (model MEK 5108 K, Nihon Kohden Corporation, 1-31-4 Nishiochiai, Shinjuku-ku, Tokyo 161-8560, Japan). Hemoglobin (Hb) was measured photometrically with the HemoCue analyzer (HemoCue, Prospect Diagnostics, Viking Court, 31 Princess Road, Dronfield, Derbyshire, S18 2LX, United Kingdom) with microcuvettes preloaded with reagent. Packed cell volume (PCV) was obtained with a hematocentrifuge by using plain capillary tubes sealed with Critoseal (Krackeler Scientific, Inc., P.O. Box 1849, Albany, New York 12,201–1849, USA). Samples were centrifuged at 10,000 g for 5 min and read manually with a hematocrit

reader. The mean corpuscular volume, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin were calculated from these red cell values. White blood cell (WBC) counts were determined manually by mixing 0.38 ml of 1% ammonium oxalate with 0.02 ml of EDTA blood and placing this on a rotor for approximately 5 min. The resultant mixture was used to fill the counting chamber of an improved Neubauer hemocytometer and allowed to stand for 5 min in a moist box to allow the cells to settle. Counts were performed with phase contrast microscopy with the 340/0.65 phase objective. A total of four large squares (64 small squares) was counted; total number was divided by 20 in order to obtain WBC X 10^9/L. Blood smears were stained with May-Grunwald-Giemsa. The differential count was based on 100 counted leucocytes. At the same time, thrombocytes were counted manually. For all samples, thrombocytes were estimated to be adequate, and none were assessed as having a thrombocytopenia.

Biochemical analyses

Samples in lithium heparin were centrifuged at 3000 rpm for 10 min on arrival at the laboratory, and the plasma was separated for biochemical analysis. We kept plasma stored frozen at − 24 °C until analysis. We made 13 determinations in sampled birds: albumin (Bromocresol green method), globulin (Biuret method), urea (Urease–GLDH method), glucose (GOD-PAP method), cholesterol (Chol. esterase chol. oxidase–Trinder method), calcium (o-cresolphthalein complexone reaction), aspartate aminotransferase AST (IFCC technique), creatinine kinase CK (IFCC technique), uric acid (Uricase–Trinder method), lactate dehydrogenase LDH (pyruvate–lactate method), sodium (indirect ion selective electrode) and potassium (indirect ion selective electrode). Protein metabolism or catabolism affects levels of urea and uric acid. Cholesterol is associated with fat metabolism (Garcia-Rodriguez et al. 1987a; Alonso-Alvarez et al. 2003). Calcium levels vary according to ossification process (Viñuela et al. 1991; Dobado-Berrios and Ferrer 1997), and creatinine kinase is related to muscular activity (Alonso-Alvarez et al. 2003). LDH is useful in diagnosing tissue damage. As far as we know, normal values for all these parameters in the white-tailed sea eagle have never been previously established.

All analyses were conducted on the ILAB 600 (Instrumentation Laboratory UK Ltd., Kelvin Close, Birchwood Science Park, Warrington, Cheshire, WA3 7 PB, United Kingdom) according to manufacturer instructions. For some samples, there was insufficient sample volume to permit analysis of all the biochemical analytes. The laboratory's quality control was to run daily internal quality control material and to peer review the results with other laboratories by using two external quality assurance schemes—the Randox International Quality Assessment Scheme and the American Veterinary Laboratory Association.

Statistical analyses

All data are expressed as mean ± standard deviations (SD). Normality in distribution of variables was univariate tested and log transformed when necessary to meet normality. We used MANOVA analyses to look for the effect of sex on blood hematological and biochemistry parameters. Regression analyses were used to study relationship between age at sampling or hatching date, with blood. For analyses, we standardized hatching dates using the earliest record of hatching each year as day 1 and the difference between the earliest record and each subsequent hatching. Statistica 8.0 software statistical package was used to perform statistical procedures, and we used an alpha value of 0.05 to assess significance of results.

Ethics statement

Procedures used in this study complied with and were licensed according to laws, regulations and guidelines for all relevant activities (both in Norway and Scotland) and permits for transfer of birds between the countries. All applicable international, national, and institutional guidelines for the care and use of animals were followed. All sampling was undertaken by qualified experienced veterinarians.

Results

A total of 83 nestlings, 43 males and 40 females, between 41–66 days of age (mean = 54.22, SD = 5.7), were sampled. No eagles were injured because of our activities. Reference values of the selected blood parameters are shown in Table S1. Significant differences between sexes were found (MANOVA, Wilks statistic = 0.223, $F_{30, 19} = 2.001$, $P = 0.037$). This significant difference was determined by only some parameters. Among hematological parameters, MCH, lymphocytes *P* and thrombocytes showed significant differences between males and females. In biochemical parameters, significant differences were found only in calcium, CK and LDH (Table 1).

No effect of the age in days of the nestling when the sample was taken in any of the analyzed hematological parameters was found (multiple $r = 0.464$, $F = 0.536$, $p = 0.919$). Any significant effect on biochemical parameters were found neither (multiple $r = 0.484$, $F = 1.05$, $p = 0.424$).

Table 1 Differences by sexes (mean and SD)

Parameter	Males	Females	*F*	*p*
MCH (pg)	50.99 (0.58)	53.35 (0.69)	6.632	0.0131
Lymphocyte *P* (%)	32.19 (2.82)	23.50 (2.58)	6.354	0.0151
Thrombocytes (10^9/L)	19.65 (2.23)	26.21 (2.30)	5.851	0.0194
Calcium (mmol/L)	2.53 (0.03)	2.61 (0,02)	5.653	0.0214
CK (U/L)	1385.00 (130.07)	985.28 (79.21)	7.014	0.0109
LDH (U/L)	791.81 (30.93)	674.82 (33.64)	6.234	0.0160

Hatching date showed no relationship with blood parameters but urea. Urea was the only one showing a strong relationship with hatching date, with those nestlings hatching later in the season showing higher urea concentration ($r=0.583$, $R^2=0.341$, $p<0.0001$, Fig. 1).

Discussion

White-tailed Sea Eagle blood values comparing to other raptors

The main objective of this study was to establish a baseline range of values of plasma chemical parameters for free-living white-tailed sea eagle nestlings and to determine the amount of variation in these parameters attributable to sex, age or hatching date (Table S1). As far as we known, this is the first time that these ranges have been reported in the literature.

Hematologic values found in the white-tailed sea eagle nestlings were similar to other published reference intervals for raptor species, with the exception of PCV (Ferrer et al. 1987; Bowerman et al. 2000; Lanzarot et al. 2001; Garcia-Montijano et al. 2002; Dujowich et al. 2005; Mealey et al. 2009; Meredith et al. 2012). Mean PCV was higher in white-tailed sea eagles than other published raptor reports. The mean hemoglobin (11.98 g/dl) was similar to wild nestling Bald Eagles (*Haliaeetus leucocephalus,* Bowerman et al. 2000) and, thus, is likely to reflect the age of the birds sampled.

Compared to other birds of prey, nestlings of white-tailed sea eagles had a mean concentrations of biochemical plasma parameters that are in the interval calculated for other raptor species (*Aegypius monachus,* Villegas et al. 2002*; Aquila adalberti*, Ferrer and Dobado-Berrios 1998*; Aquila chrysaetos,* Balasch et al. 1976*; Aquila fasciata,* Balbontin and Ferrer 2002*; Aquila pennata,* Casado et al. 2002*; Buteo swainsoni,* Sarasola et al. 2004*; Gymnogyps californianus,* Dujowich et al. 2005*; Haliaeetus leucocephalus,* Mealey et al. 2009). There are no substantive differences in any of the study parameters. The consistency of interspecific reference values found among raptors may be attributed to a common evolutionary similarity in basal physiology. Interestingly, the Osprey *Pandion haliaetus* that exhibits some significant differences in levels of blood parameters with

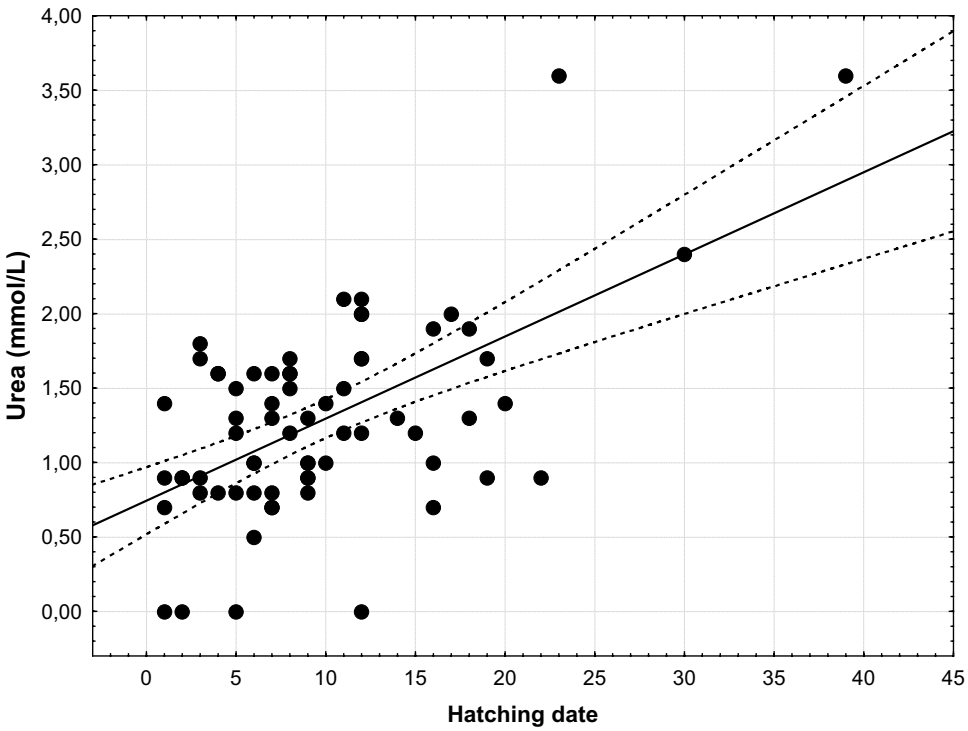

Fig. 1 Linear regression between hatching date and blood urea level in white-tailed sea eagle nestlings. A highly significant relationship was found ($r=0.583$, $R^2=0.341$, $p<0.0001$) with those nestlings hatching later in the season showing higher urea concentration (poor conditions) than earlier hatched

other raptors constitutes a monospecific family, which is clearly separated from other taxa within Falconiformes based on molecular phylogeny (Muriel et al. 2013). Although the effect of diet should be considered, other raptors, including closer relatives of WTSE, also included fish in diet but showed similar values to other raptor species preying more on small mammals and birds (Muriel et al. 2013).

Although birds, including raptors, are considered uricotelic, the mean urea concentration in white-tailed sea eagle nestlings was still double the mean concentration of uric acid, hence transformation to comparable units. This higher contribution of urea to nitrogenous waste is a phenomenon that has already been observed to occur in other raptors (e.g., Ferrer and Dobado-Berrios 1998; Casado et al. 2002; Balbontin and Ferrer 2005; Muriel et al. 2013) and fish-eating species like storks, pelicans, and herons, because of a diet rich in protein (Polo 1995).

Effects of sex and age in blood values

Sex differences had been detected more frequently in hematological parameters (red cell number, hematocrit, hemoglobin, etc.) rather than in biochemical plasma variables (e.g., Mulley 1979; Tell and Citino 1992; Ferrer and Dobado-Berrios 1998; Ferrer et al. 2017a). Nevertheless, in some species of raptors, sex-specific differences in plasma chemistry parameters in nestlings have been found, probably related with larger sexual dimorphism in size (Bonelli's Eagle *Aquila fasciata*, Balbontín and Ferrer 2002; Booted Eagle, *Aquila pennata*, Casado et al. 2002; *Pandion haliaetus*, Muriel et al. 2013). In our case, differences between sexes in MCH, thrombocytes (higher concentrations in females) and lymphocytes (higher in males) were found. Among biochemical parameters, calcium showed lower values in males and CK and LDH lower in females, suggesting a relatively worse condition and more muscular activity in males compared to females (Knuth and Chaplin 1994). Those differences could be related with differences in growth rates between sexes during the nestling period, with larger females growing faster than smaller males (Balbontin and Ferrer 2002).

In the present study, we did not find significant effects of age at sampling in any of the hematological or biochemistry parameters studied.

Hatching date and blood urea levels

High values of urea have proven to be good indicators of undernourishment (Garcia-Rodriguez et al. 1987a; Ferrer et al. 1987; Polo 1995), while total protein concentrations tend to decrease under conditions of malnutrition (Garcia-Rodriguez et al. 1987a). Triglyceride and cholesterol values are related to lipid ingestion as well as endogenous synthesis (Ferrer et al. 1987), and glucose values may decrease with prolonged food deprivation (Garcia-Rodriguez et al. 1987b). Urea is a minor pathway for protein degradation in birds but the activity of liver arginase (the enzyme on which urea production in birds depends) has increased after a prolonged fast, and the rise of urea during protein catabolism may be explained by a greater arginine availability (Garcia-Rodriguez et al. 1987a). Most birds will begin to use protein as an energy source once fat stores have been depleted; however, the point at which a bird transitions from using stored fat to muscle tissue as an energy source can also vary between species. Emperor Penguins, *Aptenodytes forsteri* (Groscolas 1982; Robin et al. 1988), will still have lipid stores when β-hydroxybutyrate levels in blood begin to decrease, possibly as an adaptation to maintain a critical level in the lipid composition of adipose tissue for thermal insulation in cold water. Similar pattern was described in the Chinstrap penguin *Pygoscelis antarctica* (Alfonso-Alvarez et al. 2003).

In the present study, we did not conduct any experiment of fasting and refeeding under controlled situations, but correlations of urea levels with hatching date clearly support the former as indicative of nutritional conditions. It has been frequently demonstrated that females reproducing earlier in the season produced larger broods with offspring in better physical condition (Klomp 1970; Newton and Marquiss 1984; Ferrer et al. 1993; Moreno et al. 1997). For example, early Spanish Imperial Eagle nestlings were better nourished than later ones (Ferrer et al. 1993; Muriel et al. 2013), and the same was found in short-toed eagles *Circaetus gallicus* (Baumbusch et al. 2021). In the present study, urea was correlated to hatching dates, with those chicks hatched at the end of the breeding season being in worse condition.

These different conditions could affect all the subsequent behavior of juveniles that start dispersal period in very different situations. For example, in Spanish imperial eagle, the maximum dispersal distances that occurs two years' later were highly correlated with nestling nutritional conditions, being longer for those young with lower urea level when they were 45 days old, i.e., in better nutritional conditions (Ferrer 1992a, 1993; Ferrer and Morandini 2017). In Black-browed Albatrosses *Thalassarche melanophrys*, for instance, physiological condition determined behavioral response of nestlings to a shy-bold continuum test, being bolder with poor nutritional conditions (Morandini and Ferrer 2019). In consequence, determination of physiological conditions should be considered in any active conservation program for WTSE, as in any replication of this reintroduction elsewhere, because this would have important consequences in dispersal distances and behavioral responses of nestlings after being released.

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Author contributions CS, RE and OS were RSPB (Scotland) project managers for the WTE east coast reinforcement project, and as part of their many duties CS and RE coordinated and assisted in the collection of nestlings in Norway, organized their transport, supervised and monitored the birds' time in the hacking facility and collected/collated essential supporting data. CS led in data collation on background data for this study. Along with CS and RE, SH, JH and AM supervised submission, the scope of samples and collation of/annual reports on laboratory results. MF conducted data analyses and led on an initial draft manuscript using some earlier material by JH and directly assisted in subsequent revisions by VM and DPW. DPW undertook analyses on birds' age and coordinated the submitted MS to which all co-authors contributed and agreed on.

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Data availability Raw descriptive statistics are provided in the Supplementary file. Requests for data on individual birds should be made to the corresponding author. Such requests will be reasonably considered

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