Factors affecting plasma biochemistry parameters and physical condition of Osprey (*Pandion haliaetus*) nestlings

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Abstract We assessed the normal values for 15 blood plasma biochemistry parameters and three indices of body condition (IBCs) in free-living Osprey (*Pandion haliaetus*) nestlings in Brandenburg (Germany). Values were compared with those of other raptors, and possible sexual and age-related differences were examined. In addition, we looked for possible relationships of habitat quality (measured in terms of foraging conditions and human disturbances) to nestling nutritional condition and productivity. Female nestlings showed higher mean urea levels and lower glucose values than males, which could be related to higher growth rates and nutrient demand of females at the end of the nestling period. Seven parameters also showed variation with age, probably relating to increasing body mass, metabolic rates, and physical activity during the pre-fledging stage. Conversely, the IBCs showed poor correlations with selected nutritional parameters, probably due to the homogeneously acceptable nutritional conditions of the nestlings. Finally, we found that Ospreys did not seem to adjust initial parental investment in relation to habitat quality, since productivity was not affected by habitat indicators. However, in large broods, but not in small ones, nestling nutritional condition improved as foraging conditions improved. These results suggest that females that are in better physical condition seem to produce more and better nourished nestlings in better foraging conditions.

Keywords Habitat quality · Index of body condition · Nutritional condition · Osprey · *Pandion haliaetus* · Plasma biochemistry

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Zusammenfassung

Einflussfaktoren auf biochemische Blutplasma-Parameter und körperliche Kondition nestjunger Fischadler (*Pandion haliaetus*)

Wir beurteilten Normalwerte für 15 biochemische Blutplasma-Parameter und drei Indizes für die Körperkondition (Indices of Body Condition—IBCs) bei freilebenden nestjungen Fischadlern (*Pandion haliaetus*) in Brandenburg (Deutschland). Die Werte wurden mit denen anderer Greifvogelarten verglichen und mögliche geschlechts- und altersabhängige Unterschiede wurden untersucht. Zusätzlich haben wir denkbare Zusammenhänge zwischen der Habitatqualität—gemessen anhand der Lage von Gebieten der Nahrungssuche und anhand menschlicher Störung—und dem Ernährungszustand der Nestlinge sowie der Reproduktion untersucht. Weibliche Nestlinge zeigten höhere Harnstoff-Durchschnittswerte und geringere Glukose-Werte als männliche, was mit einer höheren Wachstumsrate und einem höheren Nährstoffbedarf bei den jungen Weibchen gegen Ende der Nestlingsperiode zusammenhängen kann. Sieben Parameter zeigten Veränderungen abhängig vom Alter, wahrscheinlich im Zusammenhang mit der zunehmenden Körpermasse, mit dem höheren Metabolismus und mit der steigenden körperlichen Aktivität während der

Phase kurz vor dem Flüggewerden. Umgekehrt zeigten die IBCs geringe Korrelationen mit ausgewählten Parametern bezüglich der Nährstoffversorgung, wahrscheinlich aufgrund angemessener und gleichmäßiger Versorgung der Nestlinge. Schließlich fanden wir, dass Fischadler ihre ursprünglichen elterlichen Investitionen vermutlich nicht der Habitatqualität anpassten, da die Reproduktion nicht von den Habitateigenschaften beeinflusst wurde. Jedoch verbesserte sich die Nährstoffversorgung in großen Bruten mit besseren Bedingungen zur Nahrungssuche, nicht aber in kleinen Bruten. Unsere Ergebnisse legen es nahe, dass Weibchen mit besserer Körperkondition mehr und besser ernährte Jungvögel hervorbringen, insbesondere unter besseren Bedingungen für die Nahrungssuche.

Introduction

Studies of plasma biochemistry and hematology are important for veterinary diagnoses of wild and captive animals, but also in a wide range of work performed across many disciplines, such as scientific research into animal ecology or the management of endangered species. Plasma levels of biochemistry parameters provide valuable information on the physiological condition of the animal of interest, which is related to individual quality and therefore various other related factors, including survivorship, fecundity, spatial ecology, evolutionary ecology, etc. (Ferrer 1993). Since the 1970s, blood parameters have increasingly been used as physiological indicators, body condition indices have been developed, and reference values have been assessed for many species (e.g., Johnson et al. 1985; Ferrer et al. 1987; Dujowich et al. 2005). However, most of these studies have dealt with domestic or captive animals; few of them provided chemistry values for wild species. In the last 20 years, studies have also focused on the influence that factors such as gender, age, circadian and seasonal rhythms, reproductive stage, diet, body condition, genetics, fasting, stress, pathological disorders, and captivity have on physiological condition (e.g., García-Rodríguez et al. 1987a; Dobado-Berrios et al. 1998; Tilgar et al. 2004; Alonso-Álvarez 2005; Jerzak et al. 2010). However, despite the fact that the availability of biochemical indicators is increasing, there is still a lack of integration of this information as normal explicative cofactors into comprehensive analyses of biological and ecological patterns, and very few studies have applied this integrative approximation of blood chemistry, despite its utility (e.g., natal dispersal and regulation of postfledging period; Ferrer 1992a, b). Therefore, reference blood chemistry values of wild animals are required so that the effects of such factors on blood parameters can be interpreted, and in order to incorporate general physiology into ecological research and management actions.

The Osprey (*Pandion haliaetus*) is a cosmopolitan bird of prey with an ecology that has been widely studied from different points of view: development, breeding, migration, population dynamics, etc. (e.g., Poole 1989; Saurola 1997; Kjellén et al. 2001). Moreover, the Osprey has received much attention in toxicological and biomagnification studies, since it is considered a top-consumer species in aquatic ecosystems and is specialized for a fish diet (Steidl et al. 1991; Weber et al. 2003; Toschik et al. 2005; Grove et al. 2009). Despite this, there is a surprising lack of information about plasma biochemistry or hematology in Osprey, and no published values for blood parameters are available.

Some studies have analyzed the influence of environmental characteristics on breeding performance of Ospreys (e.g., Van Daele and Van Daele 1982; Löhmus 2001; Harmata et al. 2007). These works have shown that breeding Ospreys seem to adjust parental investment during the nestling and post-fledging periods in relation to habitat foraging quality, but not during the pre-laying period. However, there is little information on nestling quality in relation to habitat characteristics and the corresponding adjustment of parental care. Furthermore, the few studies that have examined variation in nestling body condition using growth rates and morphological clues failed to find relations with habitat quality (e.g., Steeger and Ydenberg 1993). Thus, appropriate plasma biochemical parameters could be used to examine the potential relationships between habitat and nestling body condition using a more straightforward approach.

Therefore, in the work described in the present paper, we (a) determined the levels of 15 selected plasma metabolites and enzyme activities in free-living Osprey nestlings, (b) discerned the effects of relevant factors such as sex and age on these variables, and (c) derived relationships between nutritional parameters and simple condition indices. We also (d) examined the potential effect that habitat quality, measured in terms of feeding conditions and human disturbances, could have on the physical condition of Osprey nestlings.

Materials and methods

Area of study

We studied a breeding population of Ospreys in the state of Brandenburg (eastern Germany, 52°15′N, 13°55′E). In the study area, the landscape is characterized by low plains (50 m a.s.l.) with a mosaic of forest plantations (*Pinus sylvestris*), remnants of broadleaf natural forests (*Quercus robur*, *Fagus sylvatica*, *Betula* sp.), agricultural lands,

freshwater lakes, and small rivers. The climate is temperate with a continental influence. Monthly average temperatures range from −1 to 18 °C (annual mean of 8.7 °C), and the mean annual rainfall is 510 mm (Deutscher Wetterdienst).

Study species

The Osprey is a large, long-lived, diurnal, and primarily migratory bird of prey that is associated with shallow waters (fresh or marine) and feeds almost exclusively on live fish (Poole 1989; del Hoyo et al. 1994). It shows a near-cosmopolitan distribution (it is not found in Antarctica), though its breeding areas are mainly the Holartic and Australasia and it has tropical and subtropical wintering grounds (Ferguson-Lees and Christie 2001). The *haliaetus* subsp. occurs in Europe, mainly in the northern and eastern regions, where there is an estimated overall population of 10,000 breeding pairs (BirdLife International 2012). Following a general worldwide decline due to direct persecution, use of pesticides, and a lack of suitable trees for nesting (Saurola 1997), the Osprey population in Germany has experienced a gradual recovery since the 1970s, with increasing population density and spatial expansion (Schmidt 2001, 2010). The German Osprey population had increased to 550 nesting pairs by 2009, with a mean productivity of 1.7 young per active nest (Schmidt 2010). This expansion has been associated with adaptation to anthropogenic environmental conditions, since 75 % of the population currently nest on pylons, and the birds are occupying more open and populated areas than they were previously (Bai et al. 2009).

Data collection

During June-July 2006, we sampled 28 Osprey nestlings, 26–46 days old, from 16 nests with brood sizes varying from one to four chicks. Nestlings were therefore in the mid-to-late nestling period, since they fledge on average when 50–55 days old in migratory populations (Stinson 1977; Poole 1989). Seven nests were on active pylons, eight were on unused pylons, and one was on a hunting hut. Young were ringed with a metal ring from the Hiddensee Bird Ringing Centre on one tarsus and a color-coded ring that could be identified from distance on the other tarsus. When the hatch date was not known, age was estimated according to plumage development and the length of the flattened wing chord (Schaadt and Bird 1993) using a metal ruler to the nearest 1 mm. We also measured body mass with 2,500 g spring scales (Pesola AG, Switzerland) to the nearest 10 g (the scale had 20 g divisions, but was read to the nearest 10 g when the measurement was equidistant between divisions), and the forearm length as an indicator of body size (from the front of the folded wrist to the proximal extremity of the ulna) using a digital caliper (Muytoyo Corp.) to the nearest 0.1 mm (Muriel et al. 2010).

Plasma parameters and sex determination

Using a hypodermic needle, 2 ml of blood were extracted from the brachial vein of the wing and collected in lithium heparin tubes to prevent coagulation. Blood samples were drawn between 10:00 and 16:00 h to minimize daily variations in biochemical parameters (García-Rodríguez et al. 1987a; Ferrer 1993). Measurements can be considered postprandial, since Osprey nestlings are fed actively during the early morning, with the fish supply provided by adults to the nest peaking at 05:00–09:00 h (Stinson 1978, Poole 1989), and sunrise occurred during the sampling period at around 04:45 h at the study area. Tubes were kept in cooling containers (+4 °C) to avoid protein denaturation and hemolysis. Plasma was separated by centrifugation (4,000 rpm, 10 min) within 8 h of extraction and was immediately stored at −40 °C until analysis. The following 15 determinations were made in each plasma sample (abbreviation is indicated), except for those without sufficient plasma: urea (urease method), uric acid (uricase method), triglycerides (lipase/glycerol kinase), cholesterol (cholesterol esterase), creatinine (creatinine iminohydrolase), glucose (glucose oxidase–peroxidase), creatine kinase (CK, enzymatic method), amylase (maltoheptaose reaction), alkaline phosphatase (ALP, cresolphthalein phosphate hydrolysis), gamma-glutamyltransferase (GGT, enzymatic method), alanine aminotransferase (ALT, enzymatic method), aspartate aminotransferase (AST, enzymatic method), potassium (K, acid–base reactions), total calcium (Ca, *o*-cresolphthalein complexone), and total protein (TP, hand-held refractometer from ATAGO). The analyses were carried out with a portable autoanalyzer (Reflotron II, Boehringer Mannheim Inc.) with the reagents recommended by Roche Diagnostics GmbH, except for two parameters—GGT and Ca—which were determined with a multichannel portable analyzer (Screen Point, Hospitex Diagnostics s.r.l.) using the reagents recommended by the company. Analyses were performed at a temperature of +37 °C. Samples with apparent hemolysis and lipemia were removed from the subsequent analysis. Analyzers were calibrated routinely using the Clean and Check kit and procedures recommended by the manufacturer (Roche). Some urea values were below the lower limit of the measurement range for the Reflotron. Those values were determined with the Screen Point analyzer, and the predicted values for the Reflotron were then calculated using a linear correlation derived from pairs of values obtained for the same samples using both the Reflotron and the Screen Point analyzer ($N = 10$; $r^2 = 0.9874$, $P < 0.0001$).

Biochemical tests were performed at the Laboratory of the Botanical Zoo of Jerez de la Frontera (Spain).

Around 50 µl of blood were also kept in Eppendorf tubes with absolute ethanol to allow the sex of each individual to be determined, following Griffiths et al. (1998). We used the primers P8 (5′-CTCCCAAGGATGAGRA AYTG-3′) and P2 (5′-TCTGCATCGCTAAATCCTTT-3′) to amplify sequences from the *CHD1-Z* gene, which is unique to females, and the *CHD1-W* gene, which occurs in both males and females. Analyses were carried out at the Laboratory of Molecular Ecology at Doñana Biological Station (CSIC, Spain).

Index of body condition (IBC)

A simple but widely applied predictor of animal body condition is body mass corrected according to a measure of size (Green 2001). We used three different approaches to obtain IBCs for Osprey nestlings: ordinary least squares regression (OLS), the reduced major axis procedure (RMA), and the scaled mass index (SMI), following Green (2001) and Peig and Green (2009, 2010). OLS and RMA are based on the residuals of a regression between the natural logarithm of body mass and the natural logarithm of a morphological measurement of structural size, while SMI is calculated by rescaling body mass in relation to relative size and a derived scaling exponent (for more details, see Peig and Green 2009). We used length of forearm as an accurate indicator of body structural size (Muriel et al. 2010).

Habitat quality

We quantified habitat quality for each of the 16 sampled nests, measuring characteristics associated with trophic conditions and human disturbance. Variables related to foraging conditions were selected based on the results of a study on the foraging behavior of breeding males in a similar area of northern Brandenburg State (Schmidt 1999). Mean distance to visited lakes from nest sites was 2.3 km, with a maximum distance of 7.3 km, and males used only the three nearest lakes in 70 % of their foraging flights. Therefore, we considered four variables: minimum distance to the nearest water body (Dist_Lake), average distance to the three nearest lakes (Dist_Lake3), and surface area of water bodies within a radius of 2.3 km (Area_2) and 7.3 km (Area_7) from the nest. Ospreys tend to use shorelines for fishing and to avoid the deeper central parts of large lakes (Saurola and Koivu 1987; Poole 1989). Hence, following the results attained by Löhmus (2001), we only considered an inner band 300 m wide from the shoreline for surface estimation purposes. Despite the fact that Ospreys are relatively tolerant of human presence (Poole 1989), we considered three more predictors to account for potential human disturbances: the distances to the nearest unpaved road (Dist_UnPRoad), paved road (Dist_PRoad), and settlement (Dist_Settl). Land features were estimated using Geographic Information System software (ArcGis 9.2 and ArcView 3.2, ESRI Inc.) and Google Earth 5.0 (Google Inc.). Digitized data on water body cover were provided by the Office of Environment, Health and Consumer Protection of Brandenburg State.

Because of partial covariation among habitat quality predictors, the original number of variables (seven) was reduced in a principal components analysis (Jambu 1991). The first and second principal components (PC1, PC2) explained 49.2 and 22.5 % of the original variance, respectively, and placed each nest in a double environmental gradient. PC1 ranged from −2.817 to 4.810 and was related to feeding conditions of the habitat, since positive values indicated increased proximity to lakes and high water body surface area around the nest (Table 1). Likewise, PC2 ranged from −1.742 to 3.175 and was associated with potential human disturbance, with positive values denoting longer distances from human infrastructures (Table 1). Therefore, positive values of both components were related to potentially higher habitat quality.

Data analysis

We used generalized linear mixed models (GLMMs) with the GLIMMIX macro of the SAS 9.2 software package to examine differences between sexes in terms of plasma metabolite levels, enzymatic activity, and body measurements. We ran a simple model for each of the 15 parameters, where the parameter was the response variable, the factor "sex" was the fixed factor, and "age" was the covariate, in order to account for possible developmental

Table 1 Contribution of each original habitat feature to the first two principal components obtained in the PCA that was performed in order to combine and reduce the number (initially seven) of descriptive variables

Code	Loading PC1	Loading PC2
Dist_Lake	**−0.856818**	−0.006017
Dist_Lake3	**−0.881020**	−0.066659
Area_2	**0.771441**	0.181342
Area7c	0.631888	0.323724
Dist_UnPRoad	**0.799172**	0.240323
Dist_PRoad	−0.279150	**0.843975**
Dist_Settl	−0.473563	**0.770328**

Absolute loading values > 0.7 are marked in bold

differences. The interaction between both explanatory variables ("sex × age") was also included in the initial model. Given that siblings from the same nest share the same genetic background, parental care, and environmental conditions, their plasma values are not independent, so we also considered "nest" as a random effect in the models to avoid pseudoreplication. We fitted the GLMMs with a normal distribution and an identity link. We checked each model for normality of residuals. When they were non-normal, the response variables were log-transformed to attain normality. Hypotheses were tested using F statistics for fixed factors and Z statistics for random effects (Littell et al. 2006). When nonsignificant ($P < 0.1$), the interaction term "sex × age" was removed from the model and only main effects were examined.

We assessed potential relationships between the three IBCs derived by rescaling body mass with respect to forearm length as body size measurements (OLS, RMA, SMI) and the levels of six plasma biochemical parameters traditionally associated with energy reserves (urea, uric acid, triglycerides, cholesterol, glucose, and total protein). Simple linear regressions were performed for each of those plasma parameters (as the response variable) and each of the IBCs (as the predictor).

Urea levels were used to check for possible relationships between the nestling physical condition and habitat quality, as measured by the two predictors derived from PCA. Urea concentration has frequently been used as an accurate indicator of nutritional state in bird species with poor fat reserves, such as birds of prey (García-Rodríguez et al. 1987a). During starvation periods, tissue proteins are actively mobilized as an energy source, leading to an increase in the nitrogenous excretion components in blood derived from protein catabolism. Hence, plasma urea values tend to increase when the bird is undernourished and recover after feeding (García-Rodríguez et al. 1987a; Alonso-Álvarez and Ferrer 2001). We ran a GLMM with "urea" as the response variable, using a normal distribution of residuals and an identity link. Normality of errors was tested. The explanatory variables were "PC1" and "PC2" as predictors of habitat feeding conditions and potential human disturbances, respectively, as well as "sex," "brood size," "hatch date" (with "1" being the earliest hatch date recorded), and "age." Given that there was only one brood and only one nest with four chicks, we re-categorized "brood size" into two levels: broods with 1–2 chicks and those with three or more nestlings. All first-order interactions of fixed factors were also included in the model. In addition, "nest" was considered a random factor to account for nonindependence among siblings. We fitted the effects of the explanatory variables to the observed data via backward selection, removing nonsignificant terms ($P < 0.1$) from the initial full model until only significant terms remained. We checked the significance of interaction terms first and then the main effect terms. When an interaction was significant, we left both main effects in the final model, even if they were not significant.

Finally, we also checked for possible effects of both environmental descriptors on the productivity of the sampled Osprey population. Two GLMMs were run using "clutch size" and "brood size" as response variables. Both variables were re-categorized into two levels as previously described for "brood size" (1–2 and 3–4 eggs/chicks, respectively), and thus a binomial distribution with a logit link was used (Littell et al. 2006). "PC1," "PC2," and their interaction "PC1 × PC2" were introduced as explanatory variables in the model. When it was not significant, the interaction was removed in order to test only for main effects of predictors.

All data are expressed as the mean ± standard deviation (SD). Minimum–maximum values (range) and reference ranges, obtained by determining 95 % confidence intervals (95 % CI), are also provided. Statistics were analyzed using the SAS 9.2 (SAS Institute Inc., Cary, NC, USA) and STATISTICA 8.0 (Statsoft 2002, Tulsa, OK, USA) software packages. Differences were considered significant at $P \leq 0.05$, unless noted otherwise.

Results

Effects of sex and age

Normal reference plasma biochemistry values for free-living male and female Osprey nestlings are shown in Table 2. Sexual differences were found for two of the fifteen plasma parameters measured after controlling for territory (nest) and age. Males presented lower urea and higher glucose levels than females. In addition, seven parameters showed significant covariation with age. Whereas urea, uric acid, triglycerides, glucose, AST, and calcium values increased with age, potassium showed a decreasing trend with age.

IBCs and plasma parameters

Body mass was positively and significantly correlated with forearm length of Osprey nestlings ($r^2 = 0.586$, $P < 0.001$). However, neither the OLS, RMA, nor SMI indices were significantly correlated with the levels of urea, uric acid, triglyceride, cholesterol, glucose, or total protein in plasma ($P < 0.05$). As significant sex differences were found for the urea and glucose levels, the factor "sex" was included in those models, but no significant relationships were found.

Table 2 Unadjusted values of 15 plasma parameters for male ($n = 18$, except for K: 17 ind.) and female ($n = 10$, except for K: 9 ind.) Osprey nestlings, and results of GLMMs with 'sex' as fixed factor, 'age' as covariate and 'nest' as random effect

Parameter	Overall			Males			Females			Fixed factors			Random effects
										Sex	Age		Nest
	Mean ± SD	Range	95 % CI	Mean ± SD	Range	95 % CI	Mean ± SD	Range	95 % CI	*P*	*β*	*P*	*P*
Urea (mg/dl)	22.34 ± 3.73	15.73–31.43	20.89–23.78	21.02 ± 2.55	15.73–26.20	19.76–22.91	24.70 ± 4.45	15.73–31.43	21.52–27.88	**0.015**	0.319	**0.026**	0.170
Uric acid (mg/dl)	11.07 ± 3.69	5.53–18.9	9.64–12.5	10.34 ± 3.72	5.35–18.90	8.5–12.2	12.39 ± 3.44	6.68–18.80	9.93–14.85	0.224	0.284	**0.047**	0.052
TP (g/dl)	2.37 ± 0.46	1.1–3.4	2.19–2.55	2.31 ± 0.26	1.7–2.6	2.18–2.44	2.47 ± 0.71	1.1–3.4	1.96–2.98	0.304	0.018	0.356	0.113
Creatinine (mg/dl)	1.94 ± 0.92	0.56–5.12	1.58–2.3	1.69 ± 0.74	0.56–3.32	1.32–2.06	2.40 ± 1.06	1.30–5.12	1.64–3.15	0.086	–0.007	0.847	0.283
Glucose (mg/dl)	326.32 ± 50.45	240–409	306.76–345.88	334.00 ± 54.44	240–409	306.93–361.08	312.5 ± 41.31	241–378	282.95–342.05	**0.051**	7.148	**0.001**	0.158
Triglycerides[a] (mg/dl)	129.11 ± 17.21	105–184	122.43–135.78	126.72 ± 14.03	105–157	119.75–133.7	133.40 ± 22.03	112–184	117.65–149.15	0.560	0.012	**0.026**	0.189
Cholesterol (mg/dl)	134.25 ± 31.68	56–207	121.97–146.54	128.94 ± 31.29	56–207	113.39–144.51	143.8 ± 31.67	110–203	121.14–166.46	0.133	–2.547	0.067	0.357
CK (U/l)	302.43 ± 185.77	20–712	230.39–374.46	285.61 ± 207.95	20–712	182.2–389.02	332.70 ± 142.35	128–566	230.87–434.53	0.921	0.348	0.965	0.055
GGT (U/l)	9.35 ± 7.26	1.1–25.1	6.54–12.17	8.12 ± 6.96	1.9–22	4.66–11.58	11.58 ± 7.63	1.10–25.10	6.12–17.04	0.668	0.445	0.080	**0.009**
ALT[a] (U/l)	6.74 ± 4.84	2.1–28.6	4.86–8.62	6.88 ± 5.80	2.1–28.6	3.99–9.76	6.49 ± 2.60	2.4–.10.1	4.64–8.35	0.412	0.179	0.396	0.066
AST[a] (U/l)	15.69 ± 7.31	6.5–39.5	12.85–18.52	15.64 ± 7.91	6.5–39.5	11.71–19.58	15.77 ± 6.48	10–29	11.13–20.41	0.526	0.696	**0.036**	0.106
Amylase (U/l)	754.39 ± 174.25	433–1,180	686.82–821.96	765.89 ± 180.83	466–1,180	675.97–855.81	733.7 ± 169.08	433–956	612.75–854.65	0.482	13.639	0.078	–
ALP (U/l)	990.64 ± 157.13	613–1,300	929.71–1,051.57	985.67 ± 170.81	613–1,300	900.73–1,070.61	999.6 ± 137.2	793–1,180	901.45–1,097.75	0.850	1.135	0.870	–
K (mEq/l)	4.16 ± 2.03	1.4–8.64	3.34–4.98	4.62 ± 2.05	1.80–8.64	3.57–5.67	3.3 ± 1.78	1.40–7.27	1.93–4.68	0.102	–0.311	**<0.001**	0.325
Ca (mg/dl)	7.27 ± 1.67	4.7–11.1	6.63–7.92	7.47 ± 1.67	4.9–11.1	6.64–8.3	6.92 ± 1.68	4.7–9.6	5.72–8.13	0.224	0.177	**0.016**	**–**
Biometrics													
Body mass (g)	1,447.21 ± 203.25	1,080–1,778	1,368.4–1,526.03	1,332 ± 139.66	1,080–1,560	1,262.55–1,401.45	1,654.6 ± 112.96	1,456–1,778	1,573.79–1,735.41	**<0.001**	14.343	**0.006**	**0.017**
Forearm (mm)	189.21 ± 9.95	164–207	185.36–193.07	185 ± 7.99	164–195	181.03–188.97	196.8 ± 8.77	181–207	190.53–203.07	**<0.001**	1.171	**0.001**	0.062

The interaction 'sex × age' is not presented since it was non-significant for all the parameters and thus removed from the final models. Slope for covariate 'age' (β) and *P*-values for all fixed (*F*-statistic) and random factors (*Z*-statistic) are shown. *P*-values ≤ 0.05 are marked in bold

[a] Variable log-transformed to attain normality of residuals

Habitat quality, nestling physical condition, and productivity

Nutritional condition, as measured via urea plasma concentration, was affected by habitat feeding conditions (PC1) (Table 3). However, this relationship was dependent on brood size, as only nestlings from large broods of three or more chicks showed lower urea values with increasing PC1 scores, while the urea levels of chicks from small broods did not vary significantly with PC1 (Fig. 1). In other words, the nutritional condition of nestlings from large broods improved as habitat trophic quality improved due to increased proximity to lakes and increased water body area. On the other hand, nestling nutritional condition was not related to human disturbances (PC2). Significant sex differences for urea remained after the samples had been corrected for possible effects of habitat and brood size, whilst age was not found to be relevant when accounting for the other explanatory variables.

Habitat feeding conditions and potential human disturbance did not affect the productivities of sampled nests, as measured via clutch size (PC1: $F_{1,13} = 1.23$, $P = 0.287$; PC2: $F_{1,13} = 0.46$, $P = 0.508$) and brood size (PC1: $F_{1,13} = 0.12$, $P = 0.730$; PC2: $F_{1,13} = 2.60$, $P = 0.131$).

Discussion

Plasma reference values

In this work, we provide the normal ranges for 15 plasma biochemical parameters in free-living Osprey nestlings (Table 2), and this is the first time that these ranges have been reported in the literature. The ranges of most plasma components of Osprey neslings overlap with those reported for nestlings of other falconiform species, but there are several notable exceptions (the "Appendix" lists biochemical values for the following species: *Aegypius monachus*, Villegas et al. 2002; *Aquila adalberti*, Ferrer and Dobado-Berrios 1998; *Aquila chrysaetos*, Balasch et al. 1976; *Aquila fasciata*, Balbontín 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. 2004; *Neophron percnopterus*, Dobado-Berrios et al. 1998; *Pseudogyps africanus*, Van Wyk et al. 1998). The Osprey nestlings in this study generally showed noticeably higher values of urea, uric acid, glucose, creatinine, and to a lesser extent triglyceride, as well as lower values of total protein and cholesterol, than those other species. Lower levels of CK and hepatic transaminase enzymes (ALT, AST, GGT) were also detected in Osprey nestlings. Depending on the species and reference work considered, the differences varied from slight to strong. For instance, mean concentrations of hepatic enzymes and CK in Ospreys were up to 10–20 times lower than they are in other raptors (i.e., *A. adalberti*, *A. fasciata*, *A. monachus*, or *H. leucocephalus*), and creatinine values were between five and eight times higher in Osprey than in most other raptors or in other nonraptorial bird species which feed on fish (e.g., *Pelecanus crispus*, Polo 1995; *P. onocrotalus*, Shmueli et al. 2000). High levels of AST and (particularly) CK in healthy birds have been related to muscle cell damage (Hotchleithner 1994), physical activity (Fourie and Hattingh 1980), capture and manipulation stress (Chalmers and Barret 1982), and body mass (Polo 1995). Hence, lower

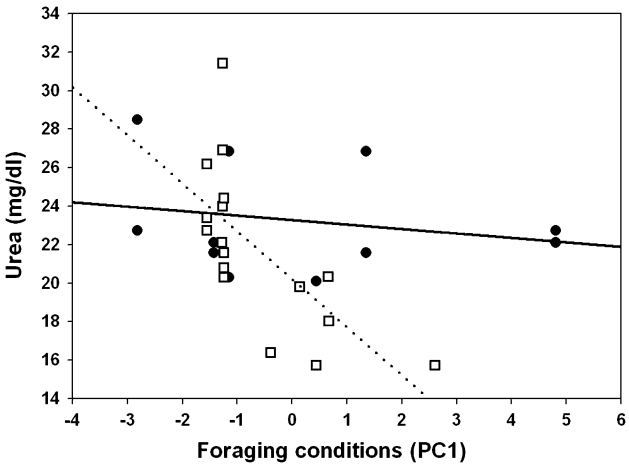

Fig. 1 Relationship between foraging conditions around the nest (PC1) and urea level in Osprey nestlings for different brood size categories (*filled circles* small broods of 1–2 chicks, *squares* large broods of 3–4 chicks). More positive values of PC1 denote larger lake surface areas and shorter distances to water bodies, and thus better feeding conditions. *Lines* represent the best linear fits for the two brood size categories (*solid line* small broods, *dotted line* large broods)

Table 3 GLMM explaining variation in urea level as an indicator of body condition in Osprey nestlings ($n = 28$) in relation to habitat characteristics derived from PCA

Explanatory variables	*df*	*F*	*Z*	*P*
Fixed effects				
Brood size	1.11	6.43		0.028
Sex	1.11	13.87		0.003
PC1	1.11	13.04		0.004
Brood size × PC1	1.11	12.75		0.004
Random effect				
Nest			1.02	0.155

Model selection was based on backward selection of significant terms from the full model, including "hatch date," "brood size," "sex," "age," "PC1," "PC2" and first interaction terms as explanatory variables. "Nest" was also considered as a random factor

levels of these enzymes in nestling Ospreys may be due to a combination of smaller body size and lower muscular stress at nestling stage in comparison to those cited species, all of which are notably larger. Other parameters such as Ca did not exhibit unusual trends compared with their values in the other species, except for *A. adalberti* and *N. percnopterus*, which showed higher calcium values. The consistent interspecific variations found may be attributed to evolutionary differences in basal physiology, since the Osprey constitutes a monospecific family that is clearly separated from other taxa within Falconiformes based on molecular phylogeny (Wink and Sauer-Gürth 2000; Lener and Mindell 2005). Additionally, the specialized diet of the Osprey, based exclusively on fish, might influence its normal plasma chemistry pattern. In any case, care must be taken when interpreting interspecific variations in biochemical values, even within the same group. Factors such as captivity, diet, physical activity, circadian cycles, and analytic methods may result in greater differences between species than expected (Ferrer 1993).

Effect of sex and age

Although birds are considered uricotelic, the mean urea concentration in Osprey nestlings was still double the mean concentration of uric acid. 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; Balbontín and Ferrer 2002) and piscivorous species like storks, pelicans, and herons, as a result of a diet rich in protein (Polo 1995). Female nestlings showed higher urea levels than males. Although the reason for this difference between the sexes is not completely clear, higher urea levels could be related to higher food demand in female nestlings as a result of higher net growth rates. In bird species with a noticeable sexual size dimorphism, like the Osprey (Schaadt and Bird 1993; Muriel et al. 2010), growth patterns may differ between sexes throughout the nestling period. Female Ospreys show higher overall growth rates than males, especially during the late nestling period from 20 to 25 days onwards, when males slow down their growth while females keep on growing until they reach their asymptotic mass (Poole 1989; Schaadt and Bird 1993). Hence, the higher food demand of females during the second half of the nestling period could explain the higher values of urea.

The trend for both urea and uric acid values to rise with nestling age may be associated with the progressive maturation of nestling renal function, which increases the clearance of nitrogen waste products (Costa et al. 1993; Dobado-Berrios et al. 1998). These increasing levels may also reflect increased protein turnover as a consequence of the rapid growth process (Dobado-Berrios et al. 1998). However, other factors such as the amount of protein ingested with the feed, individual nutritional status, and metabolic differences may influence plasma urea and uric acid levels. Future studies on age-related variations in protein catabolism and nitrogen excretion would be desirable in order to disentangle the role of parameters like urea level and uric acid level during early development in birds.

Both triglyceride concentration and cholesterol concentration are related to fat reserves, and are therefore relatively low in bird species with diets rich in protein, such as birds of prey (Griminger 1976; Polo 1995). Osprey nestlings showed rising concentrations of triglyceride with age, probably as a result of general increases in energy reserves and weight as well as a more active endogenous lipid metabolism associated with increasing physical activity during the growth stage prior to fledging (Van Wyk et al. 1998). However, we did not detect a positive correlation between cholesterol and triglycerides. A priori, a positive correlation between triglyceride concentration and cholesterol concentration would be expected, since both reflect lipid reserves. Nevertheless, Polo (1995) did not observe this positive relationship in Falconiformes, nor in other bird species.

We observed differences in plasma glucose levels between the sexes of nestling Ospreys, with males showing higher values than females. As previously noted for urea, higher overall growth rates in females during the late nestling period could lead to lower glucose levels, given that they present greater energy demands during the late growth stage, and might channel glucose reserves into tissue formation (Balbontín and Ferrer 2002). However, glucose plasma levels have shown to be highly variable because glucose is used as a rapid and immediate energy source, and depends on metabolic rate, body size, and food intake (Umminger 1977; García-Rodríguez et al. 1987b). In addition, the increase in glucose level with age observed for Osprey nestlings might be a consequence of increases in metabolic rate and weight, as already noted for several species (e.g., González and Hiraldo 1991; Ferrer and Dobado-Berrios 1998; Villegas et al. 2002; Juráni et al. 2004). In fact, as already explained for triglyceride and protein metabolism, higher glucose mobilization would be expected at the end of the nestling period, when physical activity is higher as nestlings prepare for their first flight.

Variations in transaminase enzymes like AST are difficult to interpret, since their distributions in different tissues and organs can vary among species, and may be affected by factors as diverse as muscular and soft-tissue injury, physical activity, stress, nutritional status, hydration, hemolysis, liver disease, etc. (Bell and Freeman 1971; Polo 1995). Since the Osprey nestlings analyzed did not show any apparent clinical signs of disease, the increase in AST

with age could be related to higher physical activity and flight exercises prior to fledging, which may lead to muscular stress and result in increasing AST values.

Calcium concentrations in plasma have been related to chondrogenesis, osteoblastic activity, and bone mineralization during the growth period in birds (Viñuela et al. 1991; Tilgar et al. 2004). Thus, the increasing concentrations we observed in Osprey nestlings are expected during the growth stage, with maximum values occurring at the end of structural development before fledging. However, we did not find a parallel increase in the enzyme ALP, which is associated with the elongation and ossification of skeletal structures (Viñuela et al. 1991; Tilgar et al. 2008). A similar absence of covariation in ALP with age has also been observed in other species (Costa et al. 1993; Dobado-Berrios and Ferrer 1997; Niezgoda et al. 1984). It is possible that the peak of ALP had already been attained, resulting in a plateau within the sampled age range, whereas the increase in calcium was slightly delayed to complete bone ossification. In this context, Viñuela et al. (1991) found that calcium levels reached their maximum after ALP in Red Kites (*Milvus milvus*) and Black Kites (*M. migrans*).

Muscular activity is known to increase intracellular potassium and thus to reduce its plasma concentration (Wolf et al. 1985). Hence, the decreasing potassium values observed in the Osprey nestlings with age might be related to increasing physical activity prior to fledging, as already observed in other raptors (Dujowich et al. 2005).

IBCs and plasma parameters

One of the objectives of our study was to test the relationships of certain plasma parameters employed as nutritional indicators with the estimated IBCs. High values of urea and uric acid have proven to be good indicators of undernourishment (Ferrer et al. 1987; Polo 1995), while total protein concentrations tend to decrease under conditions of malnutrition (Smith and Bush 1978). 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 (García-Rodríguez et al. 1987b). However, none of these parameters showed significant correlations with the three estimated body indices OLS, RMA, and SMI.

The general absence of a relationship between any of the selected plasma metabolites and IBCs may indicate high homogeneity in the nutritional conditions of the birds studied here, particularly given the apparent good feeding conditions of the study area. It is likely that detectable nutritional differences only arise following a minimum starvation period or when a generalized disease strikes that affects the physical state. Specific food deprivation experiments performed under controlled conditions with larger sample sizes might help us to understand the effects of nutritional stress on plasma biochemistry and IBCs in Ospreys. In any case, reported correlations between IBCs and biochemical parameters have usually been poor or irrelevant (e.g., De le Court et al. 1995; Villegas et al. 2002; Hanauska-Brown et al. 2003), since relationships between body mass and structural measurements can be masked by individual morphological variability in the absence of a significant gradient in the nutritional condition of the sampled individuals.

Habitat quality, nestling physical condition, and productivity

Our results showed that Ospreys do not tend to adjust their initial reproductive investment in relation to the foraging conditions of the surrounding habitat, since clutch size and the number of nestlings were not affected by predictors of habitat quality. Therefore, initial investment is most probably related to other factors, such as parental quality and (mainly) female physical condition before the eggs are laid, as has been shown for other species (e.g., Ferrer 1994; Vergara et al. 2010). In fact, in migratory bird species, reproductive effort may rely to some extent on post-migratory body condition, and thus on the endogenous resources stored prior to arrival (Klaassen et al. 2006). Our results are consistent with previous studies on migratory Osprey populations, which also found no correlation between clutch size or initial brood size and foraging conditions, nor between feeding rate and brood size during the nestling period (Stinson 1978; Jamieson et al. 1983; Eriksson 1986). Therefore, reduced provision of fish and lower fledgling weight would be expected in larger broods (Stinson 1977). In contrast, other studies have detected positive correlations between productivity and foraging conditions (Löhmus 2001). Interestingly, we found that nestling nutritional condition (measured via the urea level) was positively related to habitat foraging predictors, but only in large broods, not in small ones. In lower-quality habitats, nestling nutritional condition did not vary significantly with brood size, but nestlings in large broods showed lower urea values and thus better nourishment than those from small broods when feeding conditions improved. Thus, a post-hatch productivity adjustment seems to occur—at least in terms of offspring quality—in relation to habitat trophic characteristics, and is modulated by parental quality and initial investment. When food availability is high, parents in better physical condition and larger broods are able to rear more nestlings even with a better physical condition than lower-quality parents with fewer chicks. In this sense, more direct measurements of habitat productivity, like fish availability or trophic indices, would be desirable in order to obtain a more comprehensive understanding of these

relationships between habitat foraging quality and nestling nutritional condition.

Nestling body condition and productivity were not influenced by the proximity of human infrastructure. Although some previous works have found a negative relation between proximity of human activities and productivity (Van Daele and Van Daele 1982), the Osprey is considered a relatively tolerant species that has rapidly adapted to human presence. In fact, the population increase and expansion of Osprey in areas of central Europe, such as Germany, has been related to the use of pylons (which provide readily available and stable nesting structures) and the occupation of agricultural areas with more productive lakes (Bai et al. 2009), as well as the early banning of DDT and increasing ecological awareness (Schmidt 2001). Therefore, Ospreys may habituate to low-to-moderate amounts of human disturbance without suffering any negative effects on their breeding success.

Conclusions

In the present study, we obtained reference plasma values of free-living nestling Ospreys for the first time. The highly specialized fish diet and possible basal physiological variations of this particular falconiform are likely to be responsible for the observed significant differences of these values from those published for other raptor nestlings. Variations with age, which were seen for several parameters, seem to be related to increasing metabolic rates and body mass during the growth stage before fledging, which would lead to higher mobilization of metabolites linked with energy reserves and physical activity. Sexual differences in urea and glucose values would arise as a consequence of differential growth rates between genders, which are characteristic of species with sexual size dimorphism such as the Osprey. We have also shown that traditional IBCs do not have any significant correlations with selected plasma parameters associated with nutritional condition. This absence of a clear pattern could be due to high homogeneity in the (generally good) physiological conditions of the birds sampled in this work, but could also be due to the poor results that body mass residuals on size can provide, as noted in previous studies. Finally, Ospreys seem to indirectly adjust parental investment during the nestling stage. Foraging quality of the habitat may affect nestling physical condition (gauged using urea level in plasma), at least in large broods.

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Appendix

See Table 4.

Table 4 Values of plasma biochemical parameters in nestling raptors

Parameter	Booted Eagle *Aquila pennata* (Casado et al. 2002)			Egyptian Vulture *Neophron percnopterus* (Dobado-Berrios et al. 1998)			Spanish Imperial Eagle *Aquila adalberti* (Ferrer and Dobado-Berrios 1998)			Golden Eagle (1st year, captive) *Aquila chrysaetos* (Balasch et al. 1976)		
	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*
Urea (mg/dl)	20.9 ± 10.8	4.8–74.6	124	9.01 ± 4.2	1.8–16.8	127	23 ± 3	8–115	52	14 ± 2.5		2
Uric acid (mg/dl)	12.92 ± 6.54	0.2–33.47	134				11.01 ± 0.74	3.47–36.3	52			
TP (g/dl)	2.5 ± 0.1	0.1–3.3	79	2.9 ± 0.4	2.1–4	130	3.47 ± 0.06	2.17–4.59	51	1.83 ± 0.23		2
Creatinine (mg/dl)	1.07 ± 1.08	0.14–7.56	126	0.34 ± 0.14	0.2–1.4	129	0.38 ± 0.01	0.27–0.71	52			
Glucose (mg/dl)	207.01 ± 84.62	1–349	131	225.2 ± 32.43	140.52–304.47	127	243 ± 6	93–317	52	254 ± 12.7		2
Triglycerides (mg/dl)	127.86 ± 88.98	21–632	116	138 ± 35	57–239	107	108 ± 7	46–268	52			
Cholesterol (mg/dl)	211.1 ± 63	96–440	129	231.96 ± 42.53	150.77–340.21	128	208 ± 4	127–295	52			
CK (U/l)							1,320 ± 81	278–2,362	44			
GGT (U/l)							18 ± 3	0–83	52			
ALT (U/l)	9.15 ± 5.02	3.17–30.6	93	3 ± 1	0–6	44	19 ± 2	7–91	52			
AST (U/l)	86.82 ± 29.9	15.5–180	90	57 ± 17	26–107	126	175 ± 8	102–383	52			
Amylase (U/l)	636.06 ± 275.27	115–1,330	124				1,994 ± 89	1,238–4,384	52			
ALP (U/l)				1,172 ± 408	462–2,331	127						
K (mEq/l)							4.58 ± 0.69	0.82–16.9	41			
Ca (mg/dl)				2.3 ± 0.4	1.2–2.9	126	2.41 ± 0.02	2.13–2.77	52			

Parameter	Swainson's Hawk *Buteo swainsoni* (Sarasola et al. 2004)			Bonelli's Eagle (males) *Aquila fasciata* (Balbontín and Ferrer 2002)			Bonelli's Eagle (females) *Aquila fasciata* (Balbontín and Ferrer 2002)			California Condor (captive) *Gymnogyps californianus* (Dujowich et al. 2005)		
	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*
Urea (mg/dl)	17.58 ± 13.68	4–63	17	13.62 ± 3.36	7.98–19.02	13	15.12 ± 5.88	5.52–25.98	14			
Uric acid (mg/dl)	6.44 ± 2.03	3.3–10.6	17	10.89 ± 4.7	7.3–22.89	14	13.89 ± 5.17	5.4–22.89	14	4.76 ± 2.14	1.24–8.28	34
TP (g/dl)				2.98 ± 0.15	2.6–3.2	10	3.1 ± 0.28	2.7–3.6	10	3.16 ± 0.35	2.59–3.73	34
Creatinine (mg/dl)				0.28 ± 0.05	0.2–0.4	9	0.29 ± 0.04	0.24–0.37	14			
Glucose (mg/dl)				283.32 ± 37.62	217.08–333	14	252.54 ± 25.02	203.4–282.6	14	212.9 ± 38.9	148.9–277	34
Triglycerides (mg/dl)	130.35 ± 42.29	79–258	17	71.3 ± 35.1	28–166	13	81.7 ± 36.6	36–161	14			
Cholesterol (mg/dl)	282.12 ± 81.26	161–470	17	168.35 ± 23.22	118.42–214.4	16	175.31 ± 28.25	127.32–224.46	14	306.2 ± 74.7	183.4–429.1	22
CK (U/l)				3,859 ± 883	2,116–5,034	13	3,853 ± 918	1,880–5,352	14	727.8 ± 613.5	0–1,761	18
GGT (U/l)				14.5 ± 14.5	7–53	9	15.1 ± 10	5–39	12			
ALT (U/l)				11.5 ± 2.2	10–16	6	13.3 ± 3.8	9–19	8			
AST (U/l)				171.6 ± 18.2	153–202	6	203.2 ± 31.5	139–241	8	17.2 ± 13.6	0–39.6	34
Amylase (U/l)				1,148 ± 303	784–1,776	9	942 ± 194	726–1,310	12			
ALP (U/l)				2,148 ± 696	509–3,160	13	2,280 ± 327	1,696–2,759	14	469.5 ± 161.1	204.5–734.5	22
K (mEq/l)				22.3 ± 11.9	0.89–28.1	5	21.2 ± 11.7	1.15–28.6	8	3.73 ± 0.78	2.45–5.01	23
Ca (mg/dl)				8.6 ± 1	6.52–9.52	6	8.8 ± 0.92	7.2–9.8	8	10.1 ± 0.66	9.02–11.18	34

Table 4 continued

Parameter	Black Vulture (nestlings) *Aegypius monachus* (Villegas et al. 2002)			Black Vulture (1st year, captive) *Aegypius monachus* (Villegas et al. 2002)			African Whitebacked Vulture *Pseudogyps africanus* (Van Wyk et al. 1998)			Bald Eagle[a] *Haliaetus leucocephalus* (Mealey et al. 2004)		
	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*
Urea (mg/dl)	6.6 ± 3.3	3–19	57	6.2 ± 1.5	4–8	5	17.12 ± 1.36	14.94–19.18	33	13.41 ± 10.79	2–44	75
Uric acid (mg/dl)	4.8 ± 1.7	2.5–11.3	55	8 ± 4.3	3–13.9	7				13.46 ± 5.88	4.3–31.2	147
TP (g/dl)	2.9 ± 0.4	2.2–4	57	3.9 ± 0.7	3.3–5.01	7	2.93 ± 0.54	2.38–4.51	33	3.28 ± 0.98	1.4–10.4	125
Creatinine (mg/dl)							0.64 ± 0.05	0.55–0.73	33	0.24 ± 0.11	0.1–0.5	117
Glucose (mg/dl)	255.1 ± 28.2	210–349	57	292.8 ± 26.6	253–323	5	186.41 ± 31.44	129.18–276.86	33	223.03 ± 27.37	150–292	128
Triglycerides (mg/dl)	67.7 ± 56.8	15–352	56	64.7 ± 46.3	27–161	7	130.8 ± 12.18	114.55–172.13	33			
Cholesterol (mg/dl)	202.5 ± 47.6	127–384		220.6 ± 15.8	202–239	5	134.67 ± 27.43	102.84–192.18	33			
CK (U/l)										1,286.71 ± 557.69	473.2–3,190	117
GGT (U/l)												
ALT (U/l)	111.1 ± 138	2.3–419.3	57	41.84 ± 32.2	22.8–97.2	5				17.11 ± 7.41	3–42	104
AST (U/l)	102.6 ± 25	28.8–148.5	57	203.6 ± 54.4	143.7–275.5	7	78.09 ± 6.04	66.14–90.54	33	132.73 ± 48.04	46–357	129
Amylase (U/l)												
ALP (U/l)	1,336.9 ± 296.5	669–2,067	57	519.4 ± 312	189–1,057	7	274.82 ± 47.64	203.5–367.77	33	147.57 ± 51.96	35.2–322	123
K (mEq/l)	3.4 ± 0.5	2.2–4.4	41				1.29 ± 0.08	1.15–1.54	33	4.28 ± 1.82	2.2–9.7	132
Ca (mg/dl)	8.5 ± 1.7	5.7–13.9	57	10.1 ± 1.8	7.9–13	5	11.08 ± 0.53	9.98–11.92	33	9.39 ± 2.24	3.6–21.4	148

All values provided correspond to free-living individuals, except for California Condor, first-year Golden Eagles, and first-year Black Vultures, and were transformed to conventional units when necessary

[a] Urea measured as blood urea nitrogen (BUN)

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