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Are haematological parameters related to body condition, ornamentation and breeding success in wild burrowing parrots *Cyanoliseus patagonus*?

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Haematology and plasma biochemistry values are useful tools for ecological research. They have been used to investigate the physiological state and the adaptation of individuals to their habitat, changes in nutritional state of birds, body condition, the level of parasite infestation, male quality, the physical condition of nestlings, etc. In the present study we tested the role of haematological and plasma biochemistry values in burrowing parrots *Cyanoliseus patagonus* (Aves, Psittaciformes) for determining individual quality and condition. We measured triglyceride levels, plasma protein levels, plasma hue and erythrocyte sedimentation rate of nestlings and breeding adults in a colony in the north of Patagonia, Argentina. We found that plasma triglyceride levels strongly relate to changes in individual condition. Plasma levels of triglycerides were found to be strongly related to mass change, hatching order and brood size in nestlings. Levels of triglycerides were found to reflect reproductive effort in adults: males fledging larger broods had decreased levels of triglycerides. Adults with lower body condition had increased erythrocyte sedimentation rates. Plasma hue showed a strong relationship with an ornamental trait, the red abdominal patch of male adults, and with parameters of structural body size. Thus, we have shown that haematological and plasma biochemistry values, especially plasma levels of triglycerides, are good indicators of individual quality and condition in nestlings and breeding birds.

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The usefulness of haematology and plasma biochemistry as complementary veterinary tools for the diagnosis of disease, and the monitoring of the condition of birds are widely recognized. These tools are also useful in captive breeding programs for the management of endangered bird species by assessing the nutritional status of growing nestlings, or by monitoring the physiological state of individual living in different quality habitats, with different population trends or with different levels of anthropogenic disturbances (Villegas et al. 2002).

Haematology and plasma biochemistry values have also been found useful tools for ecological research (Ots et al. 1998), providing information on the physiological state and the adaptation of the individuals to the habitat. Haematology and plasma biochemistry values have been used to investigate changes in nutritional state of birds (e.g. Cherel et al. 1988, Merilä and Svensson 1995, Alonso-Alvarez and Ferrer 2001), reproductive status (e.g. Merino and Barbosa 1997), body condition (Ewenson et al. 2001), the physical condition of nestlings (Villegas et al. 2002), and health status (e.g. Moreno et al. 1998, Soler et al. 1999, Fargallo et al. 2001). Several authors used these values to establish the effects of food availability (e.g. Hoi-Leitner et al. 2001, Aquarone et al. 2002, Cucco et al. 2002). Other studies have used haematology and plasma biochemistry values in order

to study the level of parasite infestation (e.g. Potti et al. 1999, Gauthier-Clerc et al. 2003), ornamentation, male quality and sexual selection (e.g. Saino et al. 1997). Haematology and plasma biochemistry values have also proved to be useful tools for the study of moult and migration (Svensson and Merilä 1996). However, haematology and plasma biochemistry reference ranges for wild birds are still scarce due to the difficulties obtaining enough samples from wild birds.

Some simple clinical screening methods to estimate nutritional state, health, and condition of individuals include: (1) Plasma protein concentration. Total plasma protein content is considered indicative of nutritional state (e.g. Jenni-Eiermann and Jenni 1996, Ots et al. 1998). Moreover, low serum protein levels (< 2.5 g/dl) may indicate chronic disease, stress or starvation, while high (>5 g/dl) values are found during acute infections (Lewandowski et al. 1986). (2) Triglycerides. Indicators of health status and fat reserves have been shown to be associated with triglycerides (Merilä and Svensson 1995, Svensson and Merilä 1996). Higher levels of plasma triglycerides indicate a resorptive nutritional state, during which dietary fat is deposited in adipose tissues (e.g. Jenni-Eiermann and Jenni 1998). In contrast, low triglycerides levels are symptomatic of a post-resorptive, fasting state during which triglycerides from adipose tissues are hydrolysed to free fatty acids and glycerol. Jenni and Schwilch (2001) found that the absolute mass was only marginally correlated with triglycerides in reed warblers, while the change in body mass was strongly correlated with triglycerides. They concluded that feeding leads to an increase in body mass and an increase in triglycerides. They also showed that the triglycerides concentration has a diurnal rhythm, depending on the rhythm of food intake. (3) Erythrocyte sedimentation rate (SDR). SDR is a diagnostic method based on the fact that the pace of red blood cells through plasma is enhanced by increased levels of one of the major acute-phase proteins (fibrinogen) and immunoglobulins. High SDRs are indicative of many acute and chronic diseases. In serin *Serinus serinu*s nestlings, erythrocyte sedimentation rate has been found negatively correlated with food availability (Hoi-Leitner et al. 2001), while in hooded crow *Corvus corone*, low food levels induced a greater decrease in mass accompanied by an increase in erythrocyte sedimentation rate (Acquarone et al. 2002). (4) Plasma hue. It has been found in house finches *Carpodacus mexicanus* (Hill et al. 1994) that plasma hue positively correlated to plumage colouration in adult males, that the plasma hue of adult males was significantly brighter red than that of adult females, and that plasma hue differed between brighter and drabber populations of house finches.

Psittaciformes have become the most endangered order of birds in the world during the last few decades with 26% of the 350 species at risk of global extinction while another 11% are near threatened (Collar et al. 1994). The principal sources of threat arise from loss, fragmentation or degradation of breeding habitat, introduction of exotic species, persecution and hunting, and collection of birds for the live trade (Collar 1997). The increased pet trade of parrots, cockatoos, lories, conures, and macaws has, on the one hand, endangered many species in the wild by reducing their numbers quite heavily and, on the other hand, made them quite common in zoological parks and in homes as pets. Nevertheless, haematology and plasma biochemistry values of Psittaciformes are scarce and, with two exceptions (Joyner et al. 1992, Karesh et al. 1997), correspond to captive birds (e.g. García del Campo et al. 1991, Itoh 1992, Polo et al. 1998).

Burrowing parrots *Cyanoliseus patagonus* are colonial Neotropical Psittaciformes. A few haematology and plasma biochemistry values of this species have been previously studied by Polo et al. (1998). However, until now, the relationships between haematological values of burrowing parrots and parameters of adult quality and condition, breeding success, and variability caused by age, gender and hatching order of the nestling remained unstudied.

The aim of our study is to test the role of haematological and plasma biochemistry values in burrowing parrots in determining individual quality and condition. More specifically, we predicted that: (a) triglyceride levels will be related to mass change in nestlings, (b) triglyceride levels will vary with hatching order and brood size, (c) triglyceride levels will reflect reproductive effort in adults, (d) erythrocyte sedimentation rate will be related to body condition, and finally (e) plasma hue will be related with the size of adult ornaments.

Methods

Study species and site

Burrowing parrots are highly gregarious colonial Psittaciformes. In Argentina, the species occurs from the Andean slopes in the Northwest to the Patagonian steppes in the south (Darrieu 1980, Bucher and Rinaldi 1986). Burrowing parrots generally inhabit open grassland, but are also reported to occur in wooded valleys with cliffs and farmland (Juniper and Parr 1998). The breeding birds occupy the colonies 1–2 months before egg laying and leave the breeding site gradually as the young fledge. Burrowing parrots excavate their own nest burrows by tunnelling into the faces of sandstone, limestone or earth cliffs (Leonardi and Oporto 1983). Each burrow is occupied by a single pair. Burrowing parrots do not use nesting material but, rather, deposit their eggs on the sandy bottom of the nest chamber (Mey et al. 2002). Burrowing parrots lay one clutch per breeding season (Masello and Quillfeldt 2002). The

female incubates de eggs for about 24 days (de Grahl 1985) while the male provides food (Masello and Quillfeldt 2003). Clutch size varies from two to five eggs. The young hatch asynchronously with an interval of two days between subsequent nestlings. Nestlings from each brood fledged also asynchronously, with an interval of 2–3 days between nestlings (Masello and Quillfeldt 2002). Burrowing parrots have a socially and genetically monogamous breeding system with intensive biparental care (Lubjuhn et al. 2002, Masello et al. 2002, Masello and Quillfeldt 2003). The nestlings remain in the nest for about 60 days (Masello and Quillfeldt 2002).

The study was carried out from October 1999 to February 2000 in the largest and most important colony of burrowing parrots, located in a sandstone cliff at the Atlantic coast in the province of Río Negro, Patagonia, Argentina. The colony covers 9 km of cliffs (J. F. Masello unpubl. data; see also Yorio and Harris 1997), and the easternmost kilometre of the colony (41°3′S, 62°48′W) is by far the most densely populated with 6750 active nests (Masello et al. 2001). The habitat in the surroundings of the colony is primarily Patagonian steppe.

The diet of burrowing parrots of central Argentina comprises seeds and fruits, with fruits predominating during the summer (Bucher et al. 1987). In the north of Patagonia, we observed burrowing parrots eating during the breeding season seeds of the giant thistle *Carduus mariana*, the thistles *Xanthium spinosum* and *X. kravanilesii*, furthermore seeds of other wild plants such as *Avena fatua*, *Rumex crispus*, and berries of wild shrubs like *Condalia microphylla*. For the study region, Forshaw (1973) mentioned berries of *Empetrum rubrum*, *Lycium salsum* and *Discaria* sp. as part of the burrowing parrot diet, and also described the habit of burrowing parrots to feed on soft parts of plants. We observed buds and other soft vegetable matter in crop contents of nestlings especially during the first weeks of nestling rearing (November – mid-December, authors' unpubl data). Burrowing parrots can sometimes feed on grain crops, but the damage to agriculture is a locally limited phenomenon (Bucher and Rinaldi 1986).

Study methods

According to accessibility, 79 nests were selected and marked in the densest sector of the colony as part of an ongoing study of the breeding behaviour of the species. Nests were inspected every five days by climbing the cliff face. Burrowing parrots tend to desert in response of disturbance during the incubation period (de Grahl 1985) and during the first week after hatching (Masello et al. 2002). In order to reduce observer influence, nests were not disturbed until about five days after the estimated hatching date of the last nestling of a clutch. Clutch size was determined by visual inspection of the nests during incubation period, using a torch, and without capturing the adults. At the time of the first measurement, when nestlings were still clearly different sizes, the hatching rank was determined, and nestlings were individually marked. Nestlings lighter than 100 g were first marked with nail enamel on their claws. When the nestlings reached 100 g, they were banded with numbered steel bands.

When one or two adults were present, they were captured, ringed with numbered steel rings and measured. A total of 56 adults were captured at the nest: 26 males and 29 females (one individual could not be sexed). Most adults were captured in only one opportunity during the breeding season.

Blood samples (200 µl) of the adult and nestling burrowing parrots of the studied nests were taken by puncture of the brachial vein immediately after capture. Every individual was sampled once. Adults were sampled when find in the nest and nestlings were sampled between the age of 38 and 60 days. From every blood sample 150 µl were used to separate plasma from the blood cells using a blood sedimentation system (Kabe Labortechnik®, Germany). The rest of the blood was immediately suspended in 70% ethanol (Arctander 1988) and stored at 4°C for four to twelve weeks and thereafter at −20°C until processing. This blood was later used for molecular sexing of the birds. DNA was extracted using standard procedures modified according to Miller et al. (1988), for additional details see Lubjuhn and Sauer (1999). Adult and nestling burrowing parrots were sexed using PCR amplification of a highly conserved W-linked gene according to Griffiths et al. (1996), modified for Biometra®–Thermocycler T Gradient. PCR conditions were adjusted and PCR products digested with *Hae* III overnight. Fragments were separated using agarose gel electrophoresis (gel size 7×10 cm, 3% agarose, 9 V/cm).

Breeding and morphometric parameters

The following parameters of breeding success were recorded: (1) clutch size, the number of eggs laid per nest, (2) number of eggs hatched, (3) brood size, at the time of hatching of the last nestling and 30 days after, and (4) number of fledglings.

Four morphometric parameters of the nestlings and attending adults were recorded each time the nest was inspected. (1) Body mass, using a digital balance to the nearest 1 g, (2) bill length, using callipers to the nearest 0.1 mm, (3) wing length, the distance from the anterior surface of the radio carpal joint to the tip of the longest primary, to the nearest 1 mm, and (4) length of the internal tail feather to the nearest 1 mm.

Bill width was measured only in adults using callipers to the nearest 0.1 mm. This measurement was discarded for nestlings because of low repeatability due to the soft nature of the lateral parts of nestling's bill. Pre-fledging measurements of the nestlings were taken the last time they were found in the nests (i.e. one to four days before fledging). To ensure that only true pre-fledging data were included in the analyses, only data of nestlings older than 55 days were included.

Growth rates of the wing and tail of individual nestlings were determined for the linear phase of the growth curves following the method described in Masello and Quillfeldt (2002). The ages of nestlings whose hatching dates were not known were calculated from a growth curve for the bill length of known-age nestlings (Masello and Quillfeldt 2002).

Burrowing parrots have a red feather patch in the centre of the abdominal region. Using a sheet of transparent plastic the contours of the red patch of adults were copied in order to calculate the surface area, length, and width of the patch. Previous work has shown this measurements to be significantly repeatable. The red patch was copied the first time the adults were captured in each year. The red abdominal patch has been identified as a signal of individual adult quality (Masello and Quillfeldt 2003).

Haematological parameters and plasma biochemistry values

Four haematological and plasma biochemistry values were measured in plasma samples of adult and nestling burrowing parrots: the erythrocyte sedimentation rate (in two hours), the concentration of protein and triglycerides, and plasma hue.

We used a blood sedimentation system (Kabe Labortechnik®, Germany) to determine the erythrocyte sedimentation rate (in two hours) according to the standard protocol provided with the system. Plasma protein and triglycerides were determined using standard spectrophotometric test combinations modified for small amounts of plasma (5 µl plasma per determination, provided procedures n°541 and 343, Sigma Diagnostics®). For the colorimetric determination of plasma protein concentration, total protein reagent (n°541-2, Sigma Diagnostics®) and protein standard (n°540-10, Sigma Diagnostics®) have been used, and absorbance was measured with a spectrophotometer at 540 nm. For the quantitative determination of triglycerides, INFINITY™ reagent (Sigma Diagnostics®) and Sigma Diagnostics Glycerol Standard (n°G 1394) have been used, and absorbance was measured with a spectrophotometer at 520 nm. Plasma hue was scored in a scale of 1 to 5, against colour chips of Baumanns Farbtonekarte®, where 1 (citrin) was closer to yellow and 5 (orange) was closer to red.

Statistical procedures

As adult body mass is partly the result of structural body size and does not necessarily reflect the quantity of body reserves, we scaled body mass to body size as a condition index. To determine adult body condition, a multiple linear regression of body mass as dependent variable with wing, tarsus and bill length as predictors, was carried out ($n = 91$, $R = 0.465$, $F = 7.999$, $P < 0.001$). This allowed us to calculate an expected body mass for each combination of the three size factors as follows: expected body mass $= 0.84$ (wing length) $+ 7.77$ (tarsus length) $+ 1.64$ (bill length) $- 205.34$. Then, adult body condition was calculated as the ratio between observed body mass and expected body mass (see also Masello and Quillfeldt 2003).

An index of nestling body condition was calculated relative to the mean mass for nestlings of each age (m_{mean}), using the following formula: $BC = m \times 100 / m_{mean}$. This index varied between 48 and 137. Two of the four haematological parameters and plasma biochemistry values varied between nests (ANOVA, for erythrocyte sedimentation rate (in two hours): $F_{39,66} = 1.984$, $P = 0.007$; for triglyceride levels: $F_{39,66} = 1.627$, $P = 0.040$). The remaining two parameters did not vary between nest (ANOVA, for the concentration of protein: $F_{39,66} = 1.476$, $P = 0.081$; for plasma hue $F_{39,66} = 1.307$, $P = 0.167$). For the latter two parameters we used all nestlings as independent data points.

All analyses of nestling growth included only nestlings that survived to fledging. Following Krebs (1999), nestlings were classed in three categories: first, middle, and last hatched for the analyses. In nests with four nestlings, second and third hatched siblings were considered middle hatched, while in nests with five nestlings, second, third and fourth hatched siblings were considered middle hatched. Although this method could miss some differences between second, third and fourth hatched siblings, it allow comparisons between broods of different sizes. Data were analysed using Sigma Stat 2.03 and SPSS 10.0. Parametric statistical procedures were used for comparison of condition indices, except when the assumption of normality of data was violated (in which case non-parametric tests were used). Throughout this study all means are given $\pm$SE. The significance level used is $P < 0.05$. Note that sample sizes for different analyses varied as not all measurements could be taken on all birds, and because in the case of one adult bird gender could not be determined.

Results

Nestlings – body mass, mass change and body condition

Nestlings in nests with larger mean mass change (i.e. difference in mass between day of sampling and the previous measurement) had increased mean triglyceride levels ($R = 0.372$, $n = 37$ nests, $P = 0.023$). In order to test if triglyceride levels also varied within broods, we constructed a General Linear Model (triglyceride levels as the dependent variable, mass change as a covariate, nest as a fixed factor, effect of mass change: $F = 16.740$, $t = 4.092$, $P < 0.001$, effect of nest: $F = 2.115$, $P = 0.004$). In addition, the mean erythrocyte sedimentation rate per nest was correlated with the nestling body condition ($R = -0.342$, $n = 40$, $P = 0.031$). The total plasma protein content and plasma hue were not significantly correlated with the body mass or body condition of nestlings.

Nestlings – influence of hatching order and differences between families

The relationship between the hatching order of nestling burrowing parrots and body mass, body condition (body mass corrected for age) and haematological parameters, controlled for differences between nests, was studied in 22 broods using General Linear Models (GLM).

The hatching order of the nestlings did not influence body mass in a brood but differences between nests were highly significant (GLM for body mass as dependent variable, nestling hatching order as covariate, nest as fixed factor, effect of nestling hatching order: $F = 0.604$, $t = 0.777$, $P = 0.442$, effect of nest: $F = 2.826$, $P < 0.001$). The body condition of nestlings was greatly influenced by the hatching order, decreasing significantly from first, to middle, to last hatched nestlings (Fig. 1), and also differed significantly between nests (body condition as dependent variable, nestling hatching order as covariate, nest as fixed factor, effect of nestling hatching order: $F = 15.866$, $t = 3.983$, $P < 0.001$, effect of nest: $F = 4.811$, $P < 0.001$).

Triglycerides levels increased from first, to middle, to last nestlings of a brood (Fig. 1), with significant differences between families (plasma triglycerides as the dependent variable, nestling hatching order as a covariate, nest as a fixed factor, effect of nestling hatching order: $F = 5.747$, $t = 2.397$, $P = 0.021$, effect of nest: $F = 2.003$, $P = 0.027$). The total plasma protein concentration was not influenced by the hatching order and the differences observed between nests were not significant (total plasma protein as dependent variable, nestling hatching order as covariate, nest as fixed factor, effect of nestling hatching order: $F = 0.430$, $t = 0.656$, $P = 0.516$, effect of nest: $F = 1.801$, $P = 0.051$). Differences in plasma hue between chicks of different hatching order and between families were not significant. The erythrocyte sedimentation rate showed significant differences between broods but not between nestlings of different hatching order (erythrocyte sedimentation rate as dependent variable, nestling hatching order as covariate, nest as fixed factor, effect of nestling hatching order: $F = 0.041$, $t = 0.203$, $P = 0.840$, effect of nest: $F = 1.934$, $P = 0.033$).

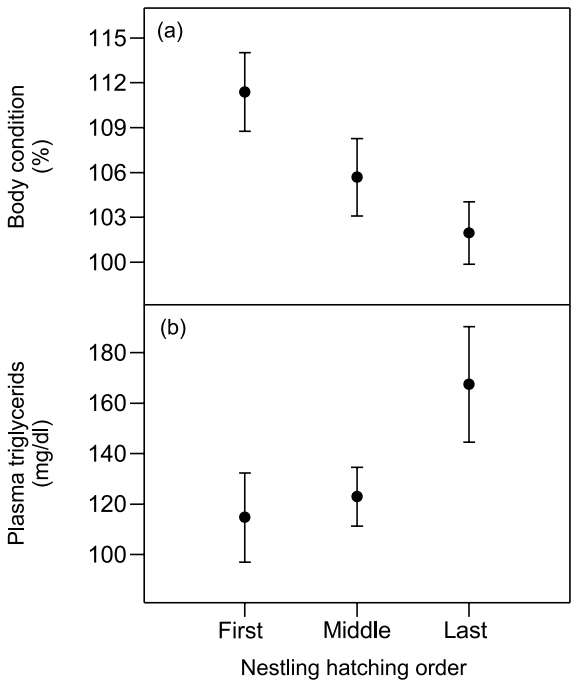

Fig. 1. Variation in body condition (a), and mean plasma triglycerides (b), between burrowing parrot nestlings of different hatching order ($n = 22$ broods).

In addition to these results, we found a correlation between triglyceride levels of first hatched nestlings and the brood size ($R = -0.412$, $n = 26$, $P = 0.037$), indicating that first hatched nestlings of larger broods showed lower triglyceride levels.

Adults and breeding success

Males with larger broods tended to have lower levels of triglycerides, and the strength of this relationship increased from clutch size ($R = -0.400$, $n = 20$, $P = 0.081$), to hatching (number of eggs hatched: $R = -0.421$, $n = 20$, $P = 0.065$), to the brood size 30 days after the hatching of the last nestling ($R = -0.574$, $n = 12$, $P = 0.051$), and to a significant correlation at fledging (number of fledglings: $R = -0.615$, $n = 12$, $P = 0.033$). In contrast, for females there was no significant correlation between triglycerides and measures of breeding success such us clutch size, number of

eggs hatched, brood size at 30 days after the hatching of the last nestling, and number of chicks fledged. We studied the relationship between triglyceride levels within pairs a found no significant differences between females and males (paired samples test $t = 0.231$, $n = 17$, $P = 0.821$).

The total plasma protein content, plasma hue and erythrocyte sedimentation rate were not significantly correlated with measures of breeding success.

Adults – differences between sexes and correlations with morphology and ornament

There were no statistical significant differences in haematological or plasma biochemistry parameters between female and male adult burrowing parrots (Table 1).

For both sexes, there were no significant correlations between triglycerides or plasma protein levels and adult morphological parameters (tail length, wing length, bill length and width, tail covert asymmetry), body mass, or ornamentation (surface, length and width of the red feather patch in the centre of the abdominal region). For males only, we found strong significant correlations between the plasma hue and parameters of ornamentation (surface of the red abdominal patch: $R = 0.512$, $n = 20$, $P = 0.021$, length of the red abdominal patch: $R = 0.588$, $n = 20$, $P = 0.006$). Plasma hue of adult males also significantly correlated with body mass ($R = 0.519$, $n = 20$, $P = 0.019$), bill length ($R = 0.566$, $n = 20$, $P = 0.009$), bill width ($R = 0.447$, $n = 20$, $P = 0.048$). For females, the plasma hue was not significantly correlated to any of the tested parameters. The erythrocyte sedimentation rate of males was negatively correlated with the tail length ($R = -0.482$, $n = 21$, $P = 0.027$), while there were no significant correlations with other morphological or ornamental parameters. The erythrocyte sedimentation rate of females was not significantly correlated to any of the tested parameters.

Unlike morphological and ornamental parameters of adult burrowing parrots (see Masello and Quillfeldt 2003), body condition was not sexually dimorphic, and thus male and female adults were included together in the analysis. The erythrocyte sedimentation rate of adults was negatively correlated with the body condition ($R = -0.322$, $n = 42$, $P = 0.037$), while body condition was not significantly correlated with any other blood parameters.

Differences between nestlings and adults

Adult and nestling burrowing parrots showed differences in all four haematological and plasma biochemistry parameters (Table 2). Nestlings had much higher triglycerides values than adults, indicating that they were storing fat reserves. Plasma protein content, plasma hue and the erythrocyte sedimentation rate were higher in adults than in nestlings.

Relationships between haematological and plasma biochemistry parameters

The level of triglycerides strongly correlated with plasma protein content when nestlings were considered as independent data points ($R = 0.267$, $n = 106$ nestlings, $P = 0.006$). Plasma hue positively correlated with the level of triglycerides and plasma protein content (for the level of triglycerides: $R = 0.239$, $n = 106$ nestlings, $P = 0.014$; for plasma protein content: $R = 0.236$, $n = 106$ nestlings, $P = 0.015$). But no significant correlations between nestling haematological and plasma biochemistry parameters have been found when the means per nest were considered.

Adult triglycerides levels strongly correlated with plasma protein content ($R = 0.601$, $n = 50$, $P < 0.001$). Adult triglycerides levels negatively correlated with the erythrocyte sedimentation rate ($R = -0.313$, $n = 49$, $P = 0.028$).

Discussion

A number of physiological tests can be used to investigate changes in individual condition in relation to behavioural and environmental changes (e.g. Cherel et al. 1988, Jenni-Eiermann and Jenni 1998, Gauthier-Clerc et al. 2003). We show here that plasma triglyceride levels strongly relate to changes in individual condition. As we predicted, plasma levels of triglycerides were found to be strongly related to mass change in nestlings, hatching

Table 1. Natural variation of haematological parameters of male and female adult burrowing parrots.

	Females			Males			Test
	Mean ± SE	Range	n	Mean ± SE	Range	n	
Plasma triglycerides (mg/dl)	88.0 ± 9.8	28.7–233.3	23	87.7 ± 7.8	40.3–158.2	21	$t = 0.023$, $df = 42$, $P = 0.981$
Plasma proteins (g/dl)	2.6 ± 0.2	1.6–5.3	23	2.4 ± 0.2	1.3–4.1	21	$t = 0.877$, $df = 42$, $P = 0.385$
Plasma hue	1.7 ± 0.2	1–4	23	2.3 ± 0.3	1–5	21	$t = 1.755$, $df = 42$, $P = 0.087$
Sedimentation in 2 hrs (mm)	10.9 ± 2.2	5–58	24	10.6 ± 1.6	5–40	22	$t = 0.117$, $df = 44$, $P = 0.907$

Table 2. Natural variation of haematological parameters of nestlings and adults of burrowing parrots.

	Nestlings			Adults			Test
	Mean ± SE	Range	n	Mean ± SE	Range	n	
Plasma triglycerides (mg/dl)	126.9 ± 7.4	17.3–570.6	106	87.1 ± 6.2	28.7–233.3	45	$t = 3.289$, $df = 149$, $P = 0.001$
Plasma proteins (g/dl)	1.9 ± 0.04	1.1–3.6	106	2.5 ± 0.1	1.3–5.3	45	$t = 6.268$, $df = 149$, $P < 0.001$
Plasma hue	1.4 ± 0.1	1–4	106	2.0 ± 0.2	1–5	45	$t = 3.995$, $df = 149$, $P < 0.001$
Sedimentation in 2 hrs (mm)	7.8 ± 0.2	4–16	106	10.8 ± 1.4	5–58	46	$t = 3.072$, $df = 150$, $P = 0.003$

order and brood size in nestlings. Also as predicted, levels of triglycerides were found to reflect reproductive effort in adults: specifically, males fledging larger broods had decreased levels of triglycerides. As predicted, adults with lower body conditions had increased erythrocyte sedimentation rates. Furthermore, in line with our last prediction, plasma hue showed a strong relationship with an ornamental trait, the red abdominal patch of male adults. Thus, we have shown that haematological and plasma biochemistry values, especially plasma levels of triglycerides, are good indicators of individual quality and condition in nestlings and breeding birds.

Nestlings

Nestlings of burrowing parrots and other Psittaciformes have been found to accumulate large amounts of fat during their development prior to the large pre-fledging mass recession observed (Masello and Quillfeldt 2002). In the present study, nestlings with higher body masses or larger mass change had higher levels of triglycerides. As predicted, the change in body mass was more strongly correlated with triglycerides than the absolute mass, and this is related to nutritional condition. A similar result was obtained by Jenni and Schwilch (2001). In accordance with our prediction, triglyceride levels of first hatched nestlings negatively correlated with brood size. All these results are in line with the findings of studies conducted in Passeriformes (Hoi-Leitner et al. 2001, Jenni and Schwilch 2001, Aquarone et al. 2002). In addition, nestlings with higher body condition had lower erythrocyte sedimentation rate. Thus, triglycerides and erythrocyte sedimentation rate may be useful parameters in order to estimate nutritional state, health and condition of many bird species.

As we predicted, significant differences in triglyceride levels between nestlings were found when hatching order was taken into account (Fig. 1). Triglyceride levels increased from first to middle to last hatched nestlings of a brood, and varied significantly between different broods. Also the body condition of nestlings was greatly affected by the hatching order, with the higher triglycerides levels associated with the lowest body conditions, both in last hatched nestlings. In a previous study on burrowing parrots (Masello and Quillfeldt 2002) our data suggested that first hatched nestlings of a brood received more food than middle and last hatched nestlings, and that these differences in food delivery rate were responsible for some of the observed variation in growth. A similar pattern has been found for other psittaciform species (e.g. Rowley and Chapman 1991, Smith 1991, Stoleson and Beissinger 1997). The higher levels of triglycerides in last hatched nestlings indicate that last hatched nestlings were in a more resorptive nutritional state at the time of sampling and recovering from the delay in depositing adipose tissues due to initial differences in food delivery by the parents (see Masello and Quillfeldt 2002). At the time of blood sampling first and middle hatched nestlings had already attained peak mass and started the mass recession period (Masello and Quillfeldt 2002), while most of the last hatched nestlings were sampled close to their peak mass. In that previous study we also found that last hatched nestlings reached the peak mass significantly later than its first hatched siblings, which is consistent with our interpretation (Masello and Quillfeldt 2002).

Nestling burrowing parrots had lower contents of plasma proteins than adults (Table 2). Similarly, Karesh et al. (1997) found that young scarlet macaws *Ara macao* had lower levels of plasma protein than sub-adults. Joyner et al. (1992) also mentioned that plasma protein values increased with age in wild Psittaciformes. Lewandowski et al. (1986) suggested that low plasma protein (<2.5 g/dl) may indicate chronic disease, stress or starvation. Chronic infestation with parasites can also result in low plasma protein values. Cassamagnaghi (1947) found no haematozoa in blood smears of burrowing parrots but his sample size was limited. We failed to detect any endoparasites (or eggs of them) in more that 150 fecal samples from adult and nestling burrowing parrots (author's unpublished data). The level of flea (Siphonaptera, Tungidae) and chewing lice *Paragoniocotes meridionalis* (Ischnocera, Philopteridae) infestation of nestling burrowing parrots was low (for data on infestation see Mey et al. 2002) and we found no correlation of flea or chewing lice prevalence with haematological and plasma biochemistry parameters. The main ectoparasite of nestling burrowing parrots in the studied colony (northeast coastal Patagonia), the bug *Psitticimex uritui* (Hemiptera, Cimicidae, Haematosiphoninae), could not be quantified in this study but their number were very high at the time of blood sampling in almost all broods. The chronic infestation with this

haematophagous bug or blood parasites may be a reason for the low plasma protein level observed in nestling burrowing parrots. Starvation as a cause of the low protein levels must be discarded because of the high level of triglycerides measured in the same plasma samples. Further investigation of the level of bug infestation and blood parasites in nestling burrowing parrots would be desirable in order to fully understand the causes of the observed plasma protein levels.

Adults

In accordance with our predictions, male burrowing parrots attending the largest broods had the lowest triglyceride levels. Low triglyceride levels are symptomatic of a postresorptive, fasting state during which triglycerides from adipose tissues are hydrolysed to free fatty acids and glycerol. In adult burrowing parrots this state could be due to reproductive effort. This interpretation is consistent with our previous finding that female burrowing parrots attending larger broods had decreased body condition and mass compared with females with smaller broods (Masello and Quillfeldt 2003). The allocation of energy between reproduction and self-maintenance has been recognised as one of the most important trade-offs affecting variation in reproductive effort (Stearns 1992), and the present data indicate that male burrowing parrots experienced a trade-off between the production of large broods and self-maintenance.

The erythrocyte sedimentation rate negatively correlated with adult body condition in burrowing parrots. The erythrocyte sedimentation rate of male burrowing parrots negatively correlated with tail length. In a previous study (Masello and Quillfeldt 2003) we found that female burrowing parrots with better body condition fledged heavier nestlings than did females in poor condition. On the other hand, female burrowing parrots which provisioned larger broods had decreased body condition (Masello and Quillfeldt 2003). We also found that tail length of adult burrowing parrots correlated strongly with parameters of nestling growth, and female tail length also correlated positively with clutch size (Masello and Quillfeldt 2003). Thus, the erythrocyte sedimentation rate of burrowing parrots is possibly reflecting both the quality of the individuals and the level of reproductive effort, as we found in the case of the plasma triglyceride level. High levels of erythrocyte sedimentation rate are also indicative of many acute and chronic diseases. Further investigation of ectoparasite levels in adult and nestling burrowing parrots is needed.

As we predicted, plasma hue of male burrowing parrots positively correlated with the surface and length of the red abdominal patch. Red colours are very common in many parrot species. Stradi et al. (2001) found that the red colours of scarlet macaws *Ara macao* are produced by at least four non-carotenoid-based pigments (linear polyenal structure). It is thought that these pigments are widespread among Psittaciformes (R. Stradi personal communication). As in scarlet macaws, the burrowing parrot has a non-carotenoid-based red ornamental coloration (Masello and Quillfeldt 2003). Two possible biochemical pathways have been suggested for the synthesis of these non-carotenoid-based pigments but further investigation is required (Stradi et al. 2001). The red abdominal patch of burrowing parrots has been identified as a signal of individual quality: it is positively correlated with male body condition and body mass, and adults with bigger patches fledged nestlings in better conditions (Masello and Quillfeldt 2003). The increased plasma hue of bigger and more ornamented (i.e. larger abdominal patch) male burrowing parrots may be the result of a higher level of some of the metabolite precursors of the non-carotenoid-based pigments in the plasma, and may thus be an indirect measurement of individual quality. Another possibility is that the increased plasma hues of these males reflect levels of carotenoids carried in the plasma, although they are not deposited in the plumage.

The plasma hue of male burrowing parrots also correlated positively with body mass, bill length and width. This is consistent with our interpretation of the plasma hue as an indirect measurement of individual quality. In a previous study (Masello and Quillfeldt 2003), we found that parameters of structural size of male Burrowing Parrots correlated with structural characters of the nestlings: large-sized males had larger fledglings than did smaller birds.

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