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Variations in leucocyte profiles and plasma biochemistry are related to different aspects of parental investment in male and female Upland geese *Chloephaga picta leucoptera*

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ABSTRACT

The analysis of plasma biochemistry and haematology to monitor the condition of birds in the wild has been found a useful tool in ecological research. Despite biparental investment in most wild birds studied, some studies of condition indices found sex differences, and attributed these to the costs of egg formation or brooding in females or a higher contribution of males to chick rearing. We studied the natural variation of haematological and plasma biochemistry parameters (namely leucocyte, lymphocyte and heterophil counts, H/L ratio and plasma concentrations of proteins, triglycerides and carotenoids) in relation to the different measures of parental investment in males and females in the Upland goose (*Chloephaga picta leucoptera*), a socially monogamous species. We found no sex differences in haematological and most plasma biochemistry parameters, but a relation to different aspects of parental investment in breeding male and female Upland geese. H/L ratios were related to body condition and capture date in males while leucocyte counts, plasma protein and plasma carotenoid concentrations varied with clutch measures and hatching date in females. Higher H/L ratios of males in a low body condition and later in the year may reflect stress associated with the investment into the establishment and defence of the breeding territory. Females with higher clutch volumes had lower total leucocyte and lymphocyte numbers and higher levels of plasma protein. Earlier hatching dates were associated with lower numbers of all leucocyte types and higher values of plasma carotenoid concentrations. This indicates that differences in health state are reflected in reproductive performance in female Upland geese. We also found sexual differences in the repeatability of haematological and plasma biochemistry parameters between years and therefore suggest that their potential as a measure of individual quality differs between male and female Upland geese. Finally, numbers of leucocyte counts and plasma triglyceride concentrations of pair partners were significantly related. No study so far investigated these parameters in pair partners and we discuss possible reasons for our finding.

1. Introduction

Many studies assessing the relationship of condition indices and reproductive performance in wild birds have been carried out in species, where males and females share a great amount of the duties associated with chick rearing and territory defence (e.g. Kilgas et al., 2006b; Moreno et al., 1998; e.g. Moreno et al., 2002b; Shutler et al., 2004). Despite the biparental care some of the studies found sex differences in stress measures, and attributed this to the costs of egg formation (Jakubas et al., 2008; Kilgas et al., 2006a; Moreno et al., 2002b) or brooding (Hõrak et al., 1998a) in females or a higher contribution of males in the late phase of chick rearing (Jakubas et al., 2008).

Our study describes the natural variation of haematological and plasma biochemistry parameters (namely leucocyte, lymphocyte and heterophil counts, H/L ratio and plasma concentrations of proteins, triglycerides and carotenoids) in relation to the different measures of parental investment in males and females such as incubation and chick rearing in the Upland goose (*Chloephaga picta leucoptera*). Upland geese belong to the order of the sheldgeese (Tadornini), a group that resembles true geese and shows similar habits, but is more closely related to shelducks and ducks. The basic breeding biology and life-cycle of Upland geese has been studied in the Falklands from 1977 to 1980 (Summers, 1983). Upland geese are highly territorial and socially monogamous, usually returning to the same territory with the same mate every year. Take up of territories starts in August, egg laying commences in late September, and most clutches are finished by the end of October. Hatching mainly takes place between mid-October and mid-November and fledging starts when chicks are about 70 days old. Males and females differ in their specific parental roles, with males establishing and intensely defending the territory and females incubating and brooding. We predict that these differences in parental roles are reflected in haematological and blood chemistry parameters.

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The analysis of plasma biochemistry and haematology to monitor the condition of birds in the wild has been found a useful tool in ecological research, as it may give a more integrative picture of the state of an animal than condition indices based on body mass alone. Leucocyte profiles, i.e. the relative numbers of different leucocyte types in the peripheral blood, have been used to study the health and condition of birds in the wild (reviewed in Davis et al., 2008). Lymphocytes and heterophils make up the majority of white blood cells in birds. Lymphocytes are highly specific and involved in a variety of immunological functions like the modulation of the immune response and immunoglobulin production (Campbell, 1995; Hawkey and Dennet, 1989). They increase in numbers during parasitic infection (Bonier et al., 2007; Ots and Hõrak, 1998) and immunological challenges (Eeva et al., 2005). A decrease in lymphocyte numbers may either be linked to stress-induced immunosuppression (Hõrak et al., 1998b) or the absence of parasite infections. Heterophils are non-specific phagocytic cells that proliferate in response to infections, inflammation and stress (Campbell, 1995; Maxwell and Robertson, 1998) and increased numbers can be found during stress, trauma and chronic bacterial infections. The heterophil/lymphocyte ratio (H/L) is often used as stress indicator in birds (Gross and Siegel, 1983; Maxwell, 1993), that is known to increase in the presence of various stressors, such as infectious diseases or starvation.

Some simple measures of blood chemistry in relation to health and condition of individuals include plasma protein concentrations, which are considered to be linked to nutritional status in birds, with rising concentrations when dietary protein intake increases or depressed levels indicating nutritional inadequacies (Jenni-Eiermann and Jenni, 1996; Jenni-Eiermann and Jenni, 1997, 1998; Ots et al., 1998; Rodríguez et al., 2005). High values of protein may also be caused by hemoconcentration, e.g. due to dehydration. Plasma triglycerides have also been linked to health state and fat reserves (Lloyd and Gibson, 2006; Masello and Quillfeldt, 2004; Quillfeldt et al., 2004), as they reflect the deposition of lipids into adipose tissues and thereby recent nutritional changes. Fasting individuals in a post-resorptive state, where triglycerides are hydrolysed from adipose tissues generally exhibit lower values of triglycerides (e.g. Jenni-Eiermann and Jenni, 1997; Jenni-Eiermann and Jenni, 1998). A third indicator of health state is the plasma concentration of carotenoids. Carotenoids are natural pigments that cannot be synthesized by vertebrates and hence must be obtained via the diet (Brush, 1981; Fox, 1979; McGraw, 2005). They have a range of health-related functions and are known to work as antioxidants and immune-enhancers (Lozano, 1994; Olson and Owens, 1998) and both direct (plasma concentration) and indirect (plasma hue) measures of carotenoids could be linked to body condition (Mougeot et al., 2009), immunocompetence (Mougeot et al., 2007; Perez-Rodriguez et al., 2008b; Quillfeldt et al., 2004) and ornamentation (Masello and Quillfeldt, 2004; Mougeot et al., 2009).

The specific aims of the current study were

(1) to determine possible sex differences in haematological and plasma biochemistry parameters in Upland geese,
(2) to determine whether haematological and plasma biochemistry parameters are linked to sex-specific parental investment,
(3) to estimate repeatability within individuals in consecutive years and to determine possible influences on variability, and
(4) to compare haematological and plasma biochemistry parameters between pair members of Upland geese.

2. Materials and methods

2.1. Study site

The study was carried out in the New Island Nature Reserve, Falkland Islands (51°43′S, 61°17′W) from October to December 2007 and 2008. The island has been established as a nature reserve in 1970 when all livestock was removed from the island. This led to an increase in the density of Upland geese, which is now one of the highest in the Falkland Islands (Quillfeldt et al., 2005).

2.2. Field measurements and sampling

At the start of each field season we mapped nests using GPS. For each nest, we determined clutch size, measured length (L, expressed in cm) and breadth (B, expressed in cm) of each egg to the nearest 0.1 mm using callipers and weighed each egg to the nearest 0.1 g using a digital balance. Egg volume (V; in cm^3) was calculated as $V=(L\times B^2\times 0.507)$ following Furness and Furness (1981). We defined total clutch volume as the sum of the volumes of each egg in the clutch. Avian eggs decrease in density with increasing length of incubation mainly due to water loss. We estimated density loss during a preliminary study in 2005 by weighing 41 eggs (from 6 clutches) twice within a period of 3–10 days. Eggs lost an average of 0.0063 ± 0.0005 SE g/cm^3 per day. We determined expected hatching date as follows: (1) we calculated egg density (D) from egg mass M (expressed in g) and V as $D=M/V$. (2) Our 2005 data showed that hatching occurred at a mean egg density of $0.89\ g/cm^3\pm0.01\ g/cm^3$ SE ($n=14$ eggs). (3) Thus, the number of days to hatching T was estimated as $T=(D-0.89\ g/cm^3)/0.0063\ g/cm^3$. We visited nests at least once a day, starting at the estimated hatching date; all eggs hatched within 0–2 days from the estimated hatch date.

We caught adults during the period when they attended their brood (chick ages 0 to 45 days, mean 11 ± 1.4 days) using a 3×5 m whoosh net. One person herded the family of geese slowly to the catching area, and when they arrived directly in front of the furled net, the other researcher pulled the trigger. Adults were marked with individual metal rings and weighed to the nearest 10 g using a digital spring balance. Head length, culmen length, and tarsus length were measured to the nearest 0.1 mm using callipers; wing length (maximum flattened chord) was measured to the nearest 1 mm using a foot rule. A blood sample (approximately 300 μL) was collected from the brachial vein. Blood samples were kept cold (4 °C) and centrifuged within 4 h. The separated plasma was stored at −20 °C until plasma protein, triglyceride and carotenoid levels were determined (see below).

Chicks were marked individually using web-tags and weighed to the nearest 1 g using a spring balance (<300 g) or to the nearest 10 g using a digital spring balance (>300 g). We measured head length, culmen length, wing length and tarsus length (±0.1 mm) using callipers. Chick ages were determined from a growth curve for head and tarsus established from chicks of known age in 2005. For each clutch, the mean chick age and thereby mean hatching date were calculated. As hatching dates could not be determined from egg density for all pairs, we used this estimated hatching date for further analyses. Estimated hatching dates from egg measures and chick measures were highly correlated ($r=0.973$, $P<0.001$, $N=41$).

The body condition of adults was determined accounting for structural size based on a regression of body mass on the first principal component (PC1) of measurements of wing, head, bill and tarsus. Body condition was then calculated as the ratio of the observed body mass to the derived expected body mass.

2.3. Leucocyte counts

The differential leucocyte count was determined as described by Ruiz et al. (2002) by examining whole blood air-dry smears. Immediately after returning from the field (no later than 4 h after sampling), the blood sample was well shaken and a drop of blood was smeared on a glass slide, using the standard two slide wedge procedure. All samples were fixed with absolute methanol. In the laboratory, smears were stained using Giemsa stain at a 1:10 dilution, for 20 min. Differential leucocyte counts were carried out with a light microscope (1000×, magnification with oil immersion), crossing the

sample from down to up to minimize differences in the thickness of the blood smear. The differential count included relative percentages of lymphocytes (L), heterophils (H), monocytes, basophils and eosinophils, which were identified according to the criteria of Hawkey and Dennet (1989). A total of 100 leucocytes were counted per slide. Using the percentages of heterophils and lymphocytes, the H/L ratio was determined. The number of leucocytes per 10,000 erythrocytes was calculated by counting the number of erythrocytes per field and multiplying by the number of fields viewed to count 100 leucocytes (e.g. Lobato et al., 2005; Merino et al., 1999; Moreno et al., 2002a).

2.4. Plasma protein, triglycerides and carotenoids

Plasma protein and triglycerides were determined using standard spectrophotometric test combinations modified for small amounts of plasma (6 μL plasma per determination, Thermo Fisher Scientific Clinical Diagnostics).

We quantified plasma carotenoid levels following Alonso-Alvarez et al. (2004). We diluted 10 μL of plasma in 90 μL of absolute ethanol, vortexed the mixture and centrifuged it at 1500 g to precipitate flocculent proteins. The supernatant was examined in an Ultrospec 2000 (Pharmarcia Biotech) spectrophotometer and the optical density at 450 nm (maximal absorbance of lutein) was determined. We calculated plasma carotenoid concentration (μg/ml) using a standard curve of lutein (Sigma Chemicals). Each plasma sample was double-tested and the obtained values were highly correlated ($r = 0.97$, $n = 83$, $P < 0.001$). Alonso-Alvarez et al. (2004) assessed the reliability of this technique by comparing a HPLC analysis of plasma samples with colorimetric measurements. HPLC determined the presence of four carotenoids in the plasma (lutein, zeaxanthin, anhydrolutein, and β-cryptoxanthin). The total amounts of carotenoids determined by the two techniques were highly correlated; colorimetric measurements could thus be considered as representative of total plasma carotenoid concentration.

2.5. Statistical analysis

We captured and measured 34 females and 33 males in 2007 and 45 females and 36 males in 2008, of which 21 females and 12 males were measured in both seasons. Additionally, in 2008 ten females were captured twice during the season (36 ± 2 days difference between captures). We could obtain egg measurements of 28 clutches (9 in 2007, 19 in 2008). Normality was tested for each data set with Kolmogorov-Smirnov tests. If necessary (no normal distribution) we transformed variables prior to the analysis. We used natural logarithm transformation for numbers of leucocytes, lymphocytes and heterophils and plasma carotenoid concentrations. H/L ratios and chick age were normalized using a square-root transformation. For birds that were sampled more than once we used only the first measurement of each individual to avoid pseudoreplication. We tested for the effects of sex and annual variation on haematological and plasma biochemistry parameters using analysis of variance (ANOVA). Given our results (see below) we then standardized (mean = 0, SD = 1) numbers of leucocytes and lymphocytes in females separately for each year (see Hõrak et al., 2002; Ochs and Dawson, 2008).

To test whether haematological and plasma biochemistry parameters could be predicted from body condition, Julian date of capture, Julian date of hatching and chick age, we performed stepwise linear regressions, with probability of 0.05 for entry and 0.10 for removal (Ochs and Dawson, 2008) separately for males and females. For the analysis of the relationship between haematological and plasma biochemistry parameters and reproductive investment in females, we used clutch volume and mean egg volume as possible explanatory variables in stepwise regression models.

We then examined the variation in haematological and blood chemistry parameters within individuals sampled in both breeding seasons. Repeatabilities were calculated as intraclass correlation coefficients according to Lessells and Boag (1987). We tested whether the differences in haematological and blood chemistry parameters between two breeding seasons could be explained by differences in body condition, chick age, capture date and hatching date also using a series of stepwise multiple regressions (P to enter = 0.05, P to exit = 0.10). For the ten females recaptured within 2008 we tested whether the differences were related to differences in body condition and chick age.

For the comparison of haematological and plasma biochemistry parameters between pair partners we used Pearson correlations. Sample sizes differed because of missing values. Some territories were not visited before chicks hatched and hence data on clutch size and egg volumes are missing. Furthermore, not all haematological and blood biochemistry measures could be determined for all individuals in case of a low quality of blood smears or missing amount of plasma for all analyses. Statistical tests were performed in SPSS 11.0. Means are given with standard errors. Significance level was set to $P < 0.05$.

3. Results

3.1. Differences between sexes and years

Mean values of the haematological and plasma biochemistry parameters of adult Upland geese in the wild are shown in Table 1. We found lymphocytes the most abundant leucocytes of adult Upland geese (Table 1), which is in line with other studies in waterfowl (Artacho et al., 2007; e.g. Matson et al., 2006). We found no significant differences between males and females in H/L ratios, numbers of leucocytes, heterophils and lymphocytes per 10,000 erythrocytes and plasma protein and triglyceride concentrations (ANOVA, all $P > 0.1$). There was a significant difference in plasma carotenoids with females showing lower concentrations than males ($F_{1,117} = 17.884$, $P < 0.001$). There were no differences between the study years apart from the numbers of leucocytes and lymphocytes/10,000 erythrocytes in females, which were lower in 2007 than in 2008 (ANOVA, leucocytes: $F_{1,59} = 6.205$, $P = 0.016$, lymphocytes: $F_{1,59} = 7.568$, $P = 0.008$).

Table 1
Mean values of heterophils/lymphocytes ratios (H/L ratio), differential leucocyte counts and plasma biochemistry parameters of adult Upland geese in the wild. We tested for differences between the sexes and years using analysis of variance (ANOVA) of transformed values (see Statistical analysis section).

	Males			Females		
	Mean + SE	Range	n	Mean + SE	Range	n
H/L	0.44 ± 0.04	0.03–1.38	56	0.38 ± 0.03	0.02–0.96	60
Leucocytes/10,000Erythrocytes	35.59 ± 2.26	5.48–85.01	56	39.78 ± 2.51	9.55–83.98	60
Heterophiles/10,000Erythrocytes	10.13 ± 1.01	0.43–32.21	56	10.06 ± 0.89	0.71–34.43	60
Lymphocytes/10,000Erythrocytes	23.26 ± 1.41	4.18–58.89	56	27.07 ± 1.68	6.47–55.71	60
Carotenoids (μg/mL)	20.11 ± 0.68	12.71–33.76	57	16.27 ± 0.81	6.05–31.48	61
Plasma protein (g/L)	52.35 ± 1.28	31.98–87.83	58	51.76 ± 0.80	39.87–68.40	60
Plasma triglycerides (g/L)	1429.5 ± 51.7	608.2–2589.4	58	1529.7 ± 66.5	812.9–4341.8	62

3.2. Males

Stepwise multiple regression ($F_{2,56}=7.604$, $P=0.001$) suggested that H/L ratios increased significantly with the date of capture ($t=2.96$, $P=0.005$, Fig. 1b), and were higher in individuals with a lower body condition ($t=-2.60$, $P=0.012$, Fig. 1a). These relationships were driven by variation in the numbers of heterophils ($F_{2,56}=8.66$, $P=0.001$), that increased with decreasing body condition ($t=-2.96$, $P=0.005$, Fig. 1a) and later date of capture ($t=2.97$, $P=0.004$, Fig. 1b). Leucocyte and lymphocyte numbers and plasma biochemistry parameters could not be explained by any of the independent variables.

3.3. Females

Hatching date was the only variable in stepwise regressions explaining the variation in numbers of total leucocytes, heterophils and lymphocytes with all variables showing higher numbers in females with later hatching date (Fig. 2, leucocytes: $F_{1,58}=5.926$, $t=2.434$, $P=0.018$, heterophils: $F_{1,58}=5.661$, $t=2.379$, $P=0.021$, lymphocytes: $F_{1,58}=5.406$, $t=2.325$, $P=0.024$). Variation in chick age was significantly related to variation in plasma carotenoid concentration ($F_{1,58}=19.993$, $t=4.471$, $P<0.001$) with lower carotenoid concentrations in females with younger chicks. H/L ratios and plasma protein and triglyceride concentrations could not be explained by any of the independent variables in these multiple regression models.

Stepwise multiple regression also suggested that leucocyte counts decreased significantly with clutch volume (Fig. 3, $F_{1,27}=5.809$, $t=-2.410$, $P=0.023$), females that had laid larger clutches had lower numbers of circulating leucocytes. This relationship was driven by variability in numbers of lymphocytes which decreased with clutch volume (Fig. 3, $F_{1,27}=7.440$, $t=-2.728$, $P=0.011$). Also plasma protein concentration showed a trend towards higher levels in females with larger clutch volumes ($F_{1,27}=3.98$, $t=2.00$, $P=0.057$), whereas plasma carotenoid concentrations were higher in females that had laid eggs with a larger mean egg volume ($F_{1,27}=5.458$, $t=2.336$, $P=0.027$). H/L ratios, numbers of heterophils and plasma triglyceride concentrations could not be explained by variation in clutch and mean egg volume.

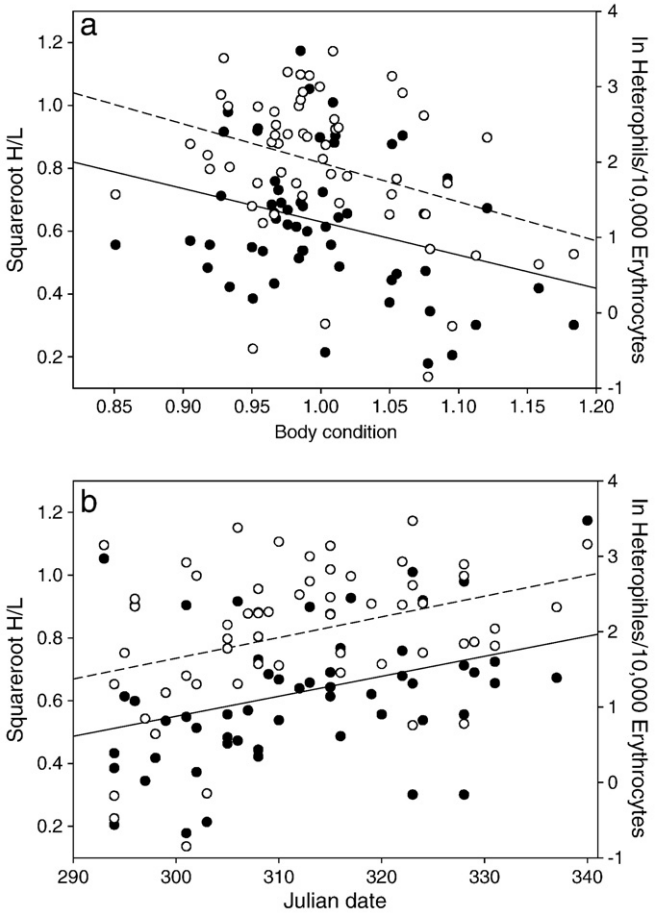

Fig. 1. Relationship between H/L ratio (black dots, solid line) and numbers of heterophils/10,000 erythrocytes (white dots, dashed line) and body condition (a) and capture date (b) of male breeding Upland geese on New Island. See text for Statistical analysis.

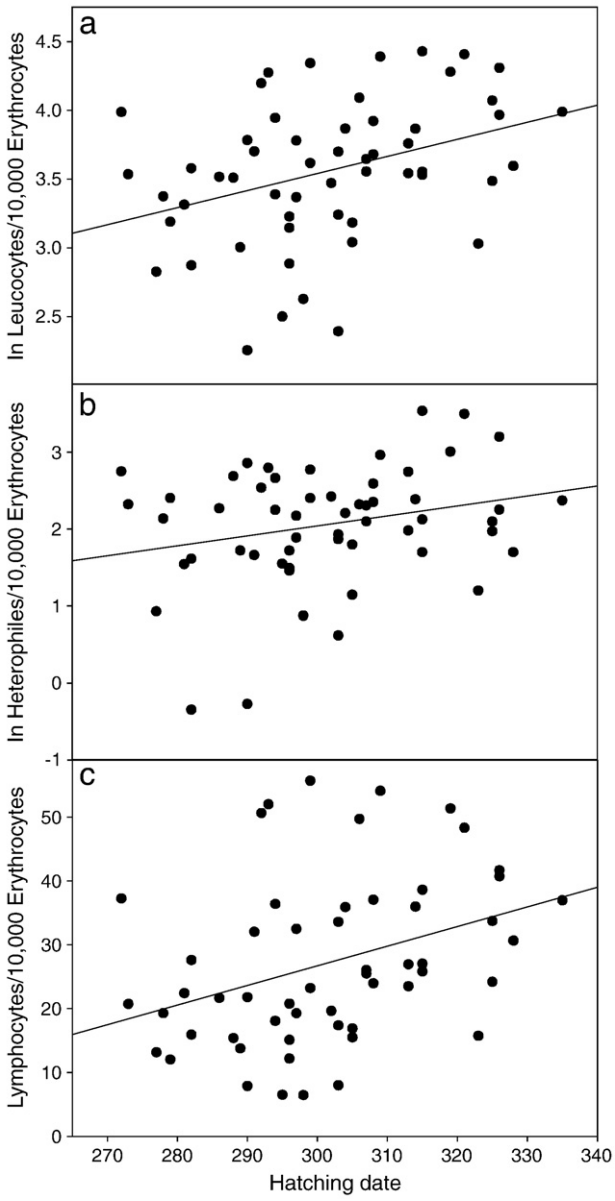

Fig. 2. Relationship between numbers of leucocytes (a), heterophils (b) and lymphocytes (c) per 10,000 erythrocytes and hatching date of female breeding Upland geese on New Island. See text for Statistical analysis.

3.4. Repeatability of haematological and plasma biochemistry parameters within individuals and influences on variability

In individual males, neither haematological nor plasma biochemistry parameters were significantly repeatable between 2007 and 2008 (all $P>0.1$). Changes of haematological and plasma biochemistry

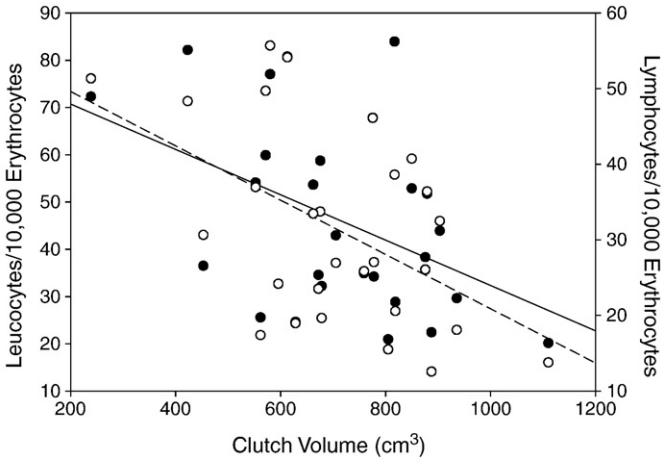

Fig. 3. Relationship between numbers of leucocytes/10,000 erythrocytes (black dots, solid line) and lymphocytes/10,000 erythrocytes (white dots, dashed line) and clutch volume of female breeding Upland geese on New Island. See text for Statistical analysis.

parameters were not influenced by changes in capture date, hatching date, chick age or body condition in multiple regression analyses (all $P>0.2$).

In individual females, only H/L ratio and plasma carotenoid concentrations showed a significant repeatability within individuals between 2007 and 2008 (H/L: $r=0.617$, $F_{18,38}=4.22$, $P=0.002$; carotenoids: $r=0.507$, $F_{18,38}=3.06$, $P=0.010$), counts of leucocytes, heterophils and lymphocytes per 10,000 erythrocytes and plasma protein and triglyceride concentrations were not significantly repeatable. Stepwise multiple regression showed that changes in leucocyte and lymphocyte counts were positively related to changes in capture date between the years (Fig. 4, leucocytes: $F_{1,17}=6.857$, $t=2.619$, $P=0.019$; lymphocytes: $F_{1,17}=7.878$, $t=2.807$, $P=0.013$). Changes in H/L ratios and heterophil counts were not influenced by changes in body condition, capture date, hatching date or chick age (all $P>0.1$). This analysis suggests that when the second sample was taken later in the year than the first sample, leucocytes and lymphocytes were more likely to be higher. Changes in plasma carotenoid concentrations were negatively related to changes in hatching date ($F_{1,17}=8.734$, $t=-2.955$ $P=0.009$), i.e. individuals with higher carotenoid concentrations in the second sample had hatched earlier. Changes in triglyceride and protein concentrations were not influenced by changes in any of the independent variables that we used in multiple regression analyses (all $P>0.2$).

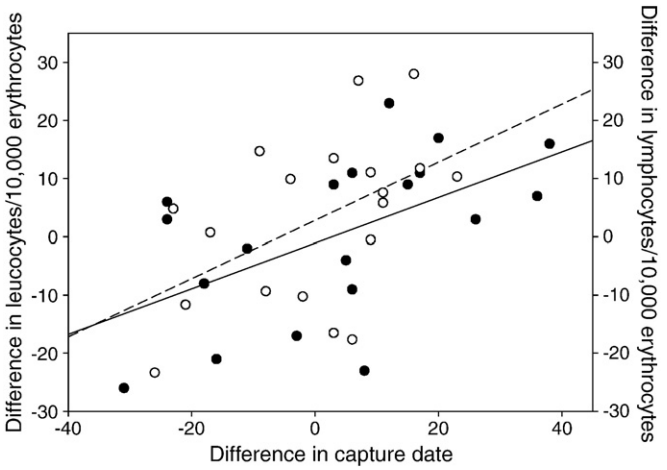

Fig. 4. Differences in numbers of leucocytes (black dots, solid line) and lymphocytes (white dots, dashed line) per 10,000 erythrocytes of female Upland geese sampled over two consecutive breeding seasons on New Island in relation to differences in capture date.

Within 2008, female individual H/L ratios, counts of heterophils per 10,000 erythrocytes and plasma protein concentrations were significantly repeatable (H/L: $r=0.658$, $F_{9,19}=4.85$, $P=0.011$, heterophils: $r=0.505$, $F_{9,19}=3.04$, $P=0.049$; plasma protein: $r=0.587$, $F_{8,17}=3.84$, $P=0.030$). We also rate plasma carotenoid concentrations as repeatable, although the p-value is slightly larger than 0.05 ($r=0.496$, $F_{9,19}=2.97$, $P=0.052$). Changes in haematological and plasma biochemistry parameters within 2008 were not significantly related to changes in chick age or body condition (all $P>0.2$).

3.5. Haematology and plasma biochemistry of pair partners

Numbers of leucocytes/10,000 erythrocytes, heterophils/10,000 erythrocytes and lymphocytes/10,000 erythrocytes of pair partners were significantly related (Pearson correlations; leucocytes: $r=0.45$, $N=57$, $P<0.001$, heterophils: $r=0.51$, $N=57$, $P<0.001$, lymphocytes: $r=0.39$, $N=57$, $P=0.002$, Fig. 5a–c). H/L was not significantly correlated in pair partners ($r=0.16$, $N=57$, $P=0.220$). Concentrations of plasma triglycerides of pair partners were significantly related, whereas plasma protein and carotenoid concentrations were not (Pearson correlations; triglycerides: $r=0.37$, $N=55$, $P=0.005$, proteins: $r=0.24$, $N=53$, $P=0.079$, carotenoids: $r=0.10$, $N=55$, $P=0.474$, Fig. 5d).

4. Discussion

4.1. Haematological and plasma biochemistry parameters and parental investment

Sex differences in haematological and plasma biochemistry parameters have been discussed to be caused by various reasons, like different endocrine profiles of males and females (Klein, 2000; Norte et al., 2009; e.g. Ots et al., 1998), differences in metabolism (Perez-Rodriguez et al., 2008a) or in parental investment (e.g. Hõrak et al., 1998a; Jakubas et al., 2008; Ots et al., 1998). We here report the case of no sex differences in haematological and most plasma biochemistry parameters, but a relation to different aspects of parental investment in male and female breeding Upland geese.

H/L ratios were related to body condition and capture date in males but not in females and this variation in H/L ratios was driven by differences in heterophil numbers. The stress related increase of H/L ratios has been shown by many studies and is a now well established tool in ecological research as this stress measure has the advantage of being less variable and longer lasting than the corticosterone stress response and multiple stressors can even have an additive effect (McFarlane and Curtis, 1989; McKee and Harrison, 1995). Higher H/L ratios in individuals in a low body condition and later in the year may reflect the investment of males into the establishment and defence of the breeding territory. Male Upland geese increase their aggressive activities gradually, besides evicting their own young from the previous breeding season, the territory has to be defended against intruding pairs looking for a breeding place and territorial boundaries with neighbouring pairs need to be maintained throughout the breeding season (Summers and McAdam, 1993). Aggressive encounters range from pacing side by side along the territorial boundaries over the exposure of their carpal knobs to gripping each other by the neck accompanied by the dashing of the wings against each other. All these activities are likely to be stressful to the birds, with low quality birds suffering more from fights than individuals in a good body condition and stress accumulating over the breeding season, which is indicated by the higher H/L ratios later in the year. Jakubas et al. (2008) also reported for Little auk (*Alle alle*) males to have increasing H/L ratios during the incubation period and attributed this to their additional activity in aggressive interactions in the colony.

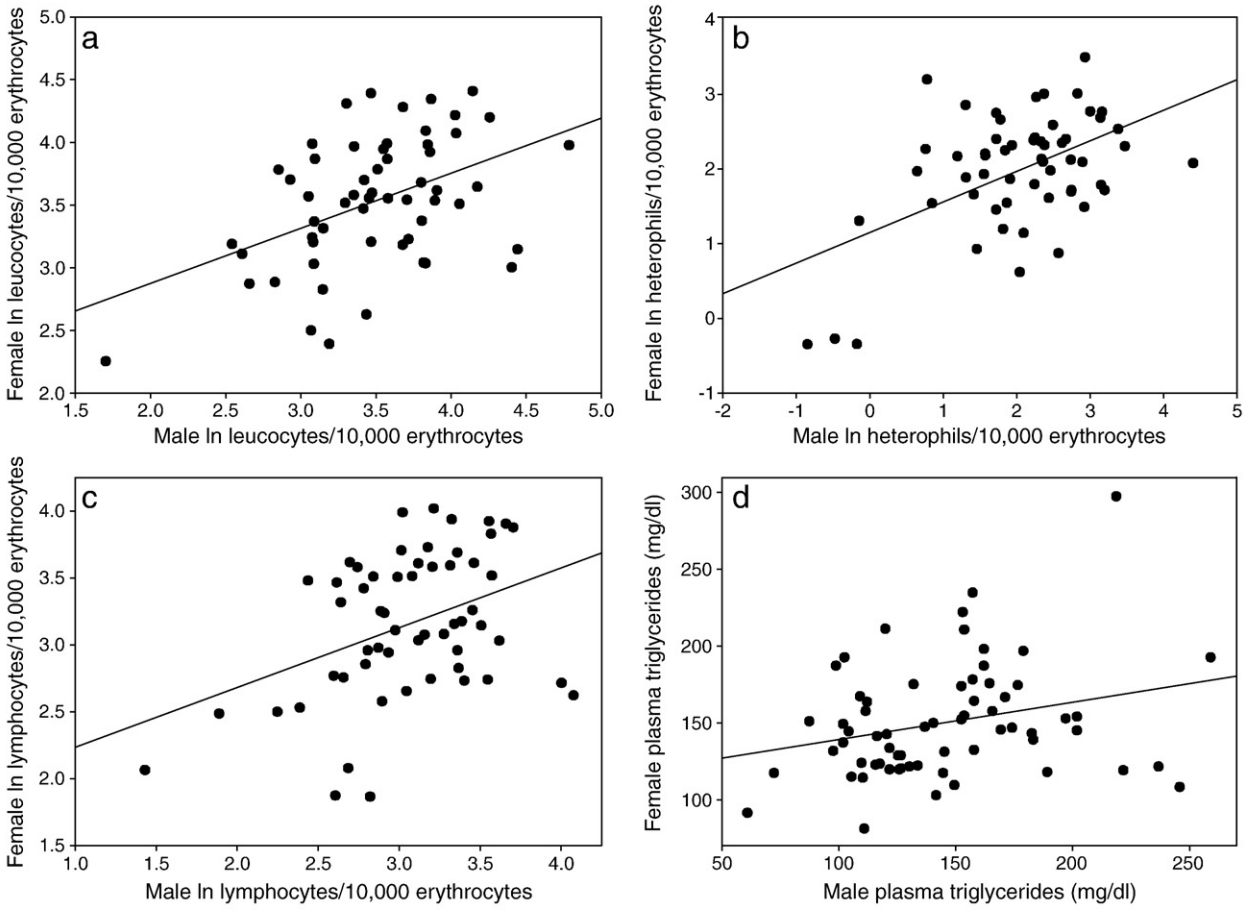

Fig. 5. Relationship of numbers of leucocytes (a), heterophils (b), lymphocytes (c) per 10,000 erythrocytes and (d) plasma triglyceride concentrations between pair partners of Upland geese on New Island. See text for Statistical analysis.

Only in female we found a relation of haematological and plasma biochemistry measures to reproductive parameters. Individuals with higher clutch volumes had lower total leucocyte numbers caused by lower numbers of lymphocytes. Poor health has been implicated as a factor which may affect reproductive performance (Gustafsson et al., 1994). As life-history theory suggests, individuals have to trade-off the investment in reproduction against the investment in body maintenance in any breeding season (Stearns, 1992). A higher exposure to infectious diseases as indicated by higher white blood cell counts and especially higher lymphocyte counts may decrease resources available for the investment into reproduction. The negative relationship we found between clutch volume and numbers of leucocytes and lymphocytes may indicate this cost of self maintenance. Low levels of circulating lymphocytes may indicate the absence of current infections requiring a specific immune response (Norris and Evans, 2000) or even low susceptibility to infections. Our results are in line with Moreno et al. (2002b) who reported that in Magellanic penguins (*Spheniscus magellanicus*) females with higher leucocyte counts layed smaller eggs and raised fewer chicks to fledging and Lobato et al. (2005) who found low leucocyte counts to be a good indicator of individual health state in Pied flycatchers (*Ficedula hypoleuca*). The lower numbers of all leucocyte types in females that hatched earlier also supports the interpretation of lower numbers as a sign for good condition and the allocation of resources into reproduction. Moreno et al. (1998) found in chinstrap penguins (*Pygoscelis antarctica*) late breeders to be in poorer health state indicated by high white blood cell counts. Early-nesting birds often lay bigger clutches; their offspring grow more rapidly and have a higher chance of survival and recruitment than late-nesting birds (Blums et al., 2002; Drent and Daan, 1980; Hochachka, 1990; Sockman et al., 2006), which results in a decrease in offspring value within a season. In the case of female Upland geese individuals fighting current infections might be allocating their resources to mount an immune response at the expense of the advantages of higher clutch size and early breeding. This is further supported by our finding that individual differences in lymphocyte numbers were related to changes in hatching date between the years, with females with higher numbers of lymphocytes in the second year hatching later. However, as haematological measures were taken after egg laying and incubation and due to the observational nature of our study it is difficult to establish causation. We therefore cannot exclude the possibility that increased reproductive effort in terms of high clutch volumes and early nesting lead to immunosuppression and therefore low lymphocyte counts (e.g. Hanssen et al., 2003; Hanssen et al., 2005; Sheldon and Verhulst, 1996). This dilemma in the interpretation of low lymphocyte counts as a sign for the lack of infections or alternatively immunosuppression has been pointed out before by Davis et al. (2008). Experimental studies including the sampling of birds before and after breeding and experiments measuring an individuals' response to an immune challenge are necessary to understand the relation of reproductive investment and haematological health parameters in this case. Also, experimentally increasing/reducing brood size could further highlight the importance of parental workload for leucocyte counts.

The analysis of plasma biochemistry also revealed that a good condition is reflected in reproductive investment in females. Plasma protein levels have been used as an indicator of body condition and

protein availability for breeding in several studies (Dawson and Bortolotti, 1997; de le Court et al., 1995; Dunbar et al., 2005) and have been found to influence clutch size (Aboul-Ela et al., 1992; Beckerton and Middleton, 1982). In female Upland geese, lower levels of plasma protein were associated with lower clutch volumes and higher lymphocyte counts and may thus indicate that these females experience stress or diseases (Lewandowski et al., 1986). Also the higher values of plasma carotenoid concentrations in birds laying bigger eggs and earlier in the season indicate that females in a better state can invest their resources into reproduction. Egg size is an important component of parental effort in birds, larger eggs may enhance offspring fitness by increasing survival prospects in the first days after hatching and competitive power through a larger offspring size and the possession of more resources to survive adverse conditions (e.g. Amundsen and Stokland, 1990; Anderson and Alisauskas, 2002; Christians, 2002; Dawson and Clark, 2000; Erikstad et al., 1998).

4.2. Repeatability of haematological and plasma biochemistry parameters

Measures of individual quality should be relatively consistent over longer time spans to be a reliable indicator for the inherent quality of individual birds (Ochs and Dawson, 2008). Hõrak et al. (2002) suggested that if haematological parameters were relatively consistent within individuals, any among-individual differences might be a reflection of differences in levels of chronic stress. Alternatively, inconsistency of these measures within individuals may suggest that levels of stress vary over time (Vleck et al., 2000). In male Upland geese, leucocyte profiles and plasma biochemistry appear to be very variable between years as we found none of these parameters to be significantly repeatable between the two consecutive breeding seasons. Together with the result that H/L ratios are related to body condition and time of the season, we can conclude that leucocyte profiles in male Upland geese can be used as a measure of current stress but not as an indicator for the inherent quality of individuals. However, in females, the significant repeatability of H/L ratios both within and between seasons indicates that these measures might be used as an index for individual quality, which is in contrast with Ochs and Dawson (2008), who found H/L ratios not repeatable between two consecutive breeding seasons in female Tree swallows (*Tachycineta bicolor*) in the wild but similar to the results of Hõrak et al. (2002) who reports a significant repeatability of H/L ratios over a 4 months period in captive greenfinches (*Carduelis chloris*) with similar intraclass correlation coefficients. Despite this repeatability supporting the use of H/L ratios as measure of inherent quality, we found no relation to other quality measures like body condition. Monitoring over several years together with a complete data set for clutch measurements, hatching dates and body condition could shed further light on the usefulness of H/L ratios as a measure of individual quality in female Upland geese.

Also plasma carotenoid concentration showed repeatability only in female Upland geese. Together with the positive relation of carotenoid concentrations with egg size this supports the use of carotenoid concentrations as a quality measure. While reproductive parameters like hatching date or clutch size might be flexible and vary according to the current condition of a bird (as indicated by their relationship to levels of plasma protein and leucocyte counts), egg size may rather be related to inherent quality. Egg volume has been discussed in the context of individual quality before, with phenotypic and genetic factors predisposing some individuals to perform better under a given set of conditions and lay larger eggs (Ardia et al., 2006; Blackmer et al., 2005; Croxall et al., 1992; Reid and Boersma, 1990). Unfortunately, until now we do not possess enough data to reliably calculate individual repeatability of egg size in Upland geese, but egg sizes of two birds of which data in different seasons exist, were significantly repeatable ($r = 0.972$, $F_{1,3} = 70.78$, $P = 0.014$).

Furthermore, we found a significant relationship of plasma carotenoid concentrations with female tarsus colouration which itself was related to reproductive investment and repeatable between years (our unpublished data). Variability between individuals even when carotenoid access and health status are standardized indicates that intrinsic physiological or genetic factors may affect the ability to absorb, transport and transform carotenoids (Hadfield and Owens, 2006; McGraw and Hill, 2001). However, because Upland geese return to the same territories year after year, territory quality and carotenoid availability in territories may be an important factor in determining the between-year correlation in plasma carotenoid levels, and we cannot exclude this possibility without experiments.

4.3. Haematological and plasma biochemistry parameters of pair partners

The correlation of numbers of leucocytes, heterophils and lymphocytes in pair partners is a surprising result and to our knowledge, no study so far investigated haematological and plasma biochemistry parameters of pairs. There are several possible explanations for this pattern. One possibility is that the correlation of these haematological parameters is a sign for mate choice with individuals in a better immunological state being mated to individuals with the same characteristics. However, as none of these count variables showed a significant repeatability between the two years on an individual base, neither in males nor in females, this explanation is less likely. A more convincing conclusion may be that these measures reflect the current condition of an individual, which still may be similar in pair partners as we caught the male and female of a pair together at the same time, thus the recent conditions have been similar for both. Also the correlation of plasma triglyceride concentrations in pair partners may be explained by the fact that both partners are dwelling the same territory and facing the same small scale conditions. Plasma triglyceride concentrations are indicative of the nutritional state and especially changes in this state and thus closely linked to current environmental conditions. Sampling during the non-breeding period when Upland geese gather in large groups around ponds and thus share the same environmental conditions, could highlight the importance of the breeding territory or recent conditions.

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