

Variation of the reed bunting (*Emberiza schoeniclus*) body condition and haematological parameters in relation to sex, age and season

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The body condition and haematological parameters of birds are influenced by a multitude of factors. Here we investigated body mass, fat scores, ratio of heterophils and lymphocytes (*H/L*), red blood cell count (RBC) of reed buntings (*Emberiza schoeniclus*), expecting differences between spring and summer (different physiological states/energy demands in breeding/post-breeding periods), sexes (different parental duties) and age groups (different experience/social status). Fat scores and size-corrected body mass were similar in all the groups. Adults in spring had higher *H/L* than in summer, which may have been due to reproduction-related stress or the seasonal elevation of glucocorticoids. The lower RBC and *H/L* in adults in summer were associated with their complete moult. Lower *H/L* in adults in summer compared to immatures may have resulted from their lower stress level (greater experience/higher social status) or a complete moult. The *H/L* ratio was similar in both sexes, despite their different parental duties.

Introduction

Birds undergo pronounced annual changes in physiology, morphology and behaviour that facilitate survival and reproduction in varying environments (Murton & Westwood 1977). This results from both changing external environmental cues and changing internal responsiveness to those cues (Dawson *et al.* 2001). Energetically demanding seasonal activities such as reproduction (Drent & Daan 1980) are often associated with a decrease in body mass (Ricklefs 1974), elevated stress level (Romero 2002) and suppres-

sion of immune function (Norris & Evans 2000).

Body condition and stress levels may be different in males and females, particularly during breeding, when one sex invests more time and/or energy in parental care than the other (Sanz *et al.* 2000, Visser & Lessells 2001). However, even in species with biparental care, males and females may differ in body condition and haematological parameters associated with stress levels because of constraints imposed by physiological, behavioural and environmental factors (e.g. Hőrak *et al.* 1998, Ots *et al.* 1998, Kilgas *et al.* 2006a, Jakubas *et al.* 2008).

Also, young and adult individuals often differ in body condition or fat reserves. Possible explanations for the lower values recorded in immature birds include less efficient foraging as a result of inexperience or lower social status and increased mobility as an antipredator strategy (immatures are more vulnerable to predation) (Woodrey & Moore 1997). Haematological parameters are often age-related: this may reflect age differences in stress level as well as in immune function and susceptibility to diseases as the blood profiles of nestling birds develop with age (Lavoie *et al.* 2007, Quillfeldt *et al.* 2008).

The reed bunting (*Emberiza schoeniclus*) is a small passerine bird, with apparent sexual dimorphism in plumage and body size (males larger than females). It inhabits tall herbage and small shrubs in marshy and swampy areas bordering fresh or brackish water. The reed bunting is socially monogamous. A pair is capable of breeding twice in a single season. The female usually lays 4–5 eggs. Both parents look after the brood. However, participation in particular parental duties differs between sexes. The female builds the nest, incubates and feeds the offspring to a greater extent than the male. Males perform part-time mate guarding prior to egg laying. They sing before pair formation and during the nesting period. It is the males that mainly feed the young after fledging. Reed buntings from central and northern Europe spend the winter in western Europe (France, Spain). Adults undergo a partial moult before breeding and a complete one before the autumn migration. Immatures undergo a partial post-juvenile moult prior to the autumn migration (Svensson 1992, Cramp 1998, Okulewicz 1989, Bouwman *et al.* 2005, Marthinsen *et al.* 2005).

The main objective of this study was to investigate whether intrinsic individual (sex and age) and/or extrinsic environmental (season) factors influence body condition and haematological parameters in reed buntings. To our knowledge, studies of haematological parameters have never been conducted in this species before. We hypothesized that the birds' body condition and haematological parameters should differ between spring and summer, reflecting the different physiological states and energy demands

of the breeding and post-breeding periods. In view of the sex differences in parental duties, we expected differences in body condition and haematological parameters between males and females. We also hypothesized that because of different experience and effectiveness in foraging, body condition and haematological parameters should differ between adults and immatures.

Materials and methods

Study site and field methods

The birds were trapped in 5–20 mist nets in reedbeds in the southern part of the “Lake Druzno” reserve (54°05'N, 19°27'E) in northern Poland. Lake Druzno is a large, shallow lake, overgrown in much of its area with reedbeds, which makes it an attractive place for breeding and moulting reed buntings. Our study was carried out in 2008, in spring (a total of 18 days of 2–3 day trapping sessions between 25 April and 28 June), corresponding to the breeding period, and summer (39 days of continuous trapping between 27 July and 4 September), corresponding to the post-breeding period, including the complete post-breeding moult of the adults, the partial post-juvenile moult of the immatures, and post-breeding dispersal (Cramp 1998). The majority of adults trapped in spring (82%) were captured at the end of April and the beginning of May, when egg laying reaches a peak in this species in Poland (Okulewicz 1989). Thus, we caught the majority of adults during the incubation period, which was confirmed by the fully-developed brood patch in all the females captured.

We trapped, ringed, aged (immature, i.e. 1-year old or adult) and sexed birds by external characters (Svensson 1992). We measured the wing length (flattened) with a ruler (± 1 mm), and body mass using an Ohaus CL 201 electronic scale (accuracy 0.1 g). We used fat scores (after Busse 2000) to estimate fat reserves. To examine haematological parameters, we took a blood sample of 10–25 μ l from each bird by brachial vein puncture (following the standard procedure used in passerines, e.g. Ots *et al.* 1998, Cuervo *et al.* 2007). We measured and blood-sampled a total of 88 reed buntings.

Parameters studied

We selected the following parameters associated with body condition and stress level: body mass, body mass corrected for body size, fat scores, leukocyte profile (proportions of heterophils, lymphocytes and their ratio — hereafter the ratio *H/L*) and numbers of red blood cells (RBC).

Body mass is a widely used and easily measured index of body condition. However, it is affected by many different factors, e.g. body size. Calculation of the body mass corrected for body size is the simplest way of separating the effects of condition from the effects of body size (Reist 1985, Krebs & Singleton 1993, Jakob *et al.* 1996, Ots *et al.* 1998) and enables fat and protein reserves to be estimated.

Fat deposits are the most important energy reserve in passerines and are usually considered a short-term indicator of condition as they vary during the day according to feeding (Bednekoff *et al.* 1994). Fat scores, based on visible subcutaneous fat on the abdomen and the furcular depression, are used to assess fat reserves. Even though the fat is deposited non-uniformly along the bird's body axis, it is deposited primarily towards the front and rear ends of the body. Thus, fat scores may be taken to be a true reflection of fat content (Hedenström *et al.* 2009).

Leukocyte profiles (relative numbers of white blood cell types) are widely used in studies to assess immune function and stress in birds (Gross & Siegel 1983, Maxwell 1993, Ruiz *et al.* 2002, Davis *et al.* 2008). Heterophils (the primary phagocytic leukocytes, proliferating in the circulation in response to infections, inflammation and stress) and lymphocytes (involved in immunoglobulin production and immune defence modulation) make up the majority (nearly 80%) of leukocytes in birds (Campbell 1995, Rupley 1997, Davis *et al.* 2008). The *H/L* ratio increases as a response to various stressors, including infectious diseases, parasite infestation, food or water deprivation, temperature extremes and psychological disturbance (Gross & Siegel 1983, Maxwell 1993, Ots & Hórak 1996, Totzke *et al.* 1999, Vleck *et al.* 2000, Ruiz *et al.* 2002, Lobato *et al.* 2005, Davis *et al.* 2008). This reaction is caused by the redistribution of lymphocytes and heterophils between the blood and other body

compartments induced by the secretion of corticosterone (Dhabhar 2002, Davis *et al.* 2008). A change in the *H/L* ratio is detectable within hours following exposure to a stressor. Thus, it is not affected by handling or blood sampling stress and is generally considered a reliable indicator of avian stress (Maxwell 1993, Davis *et al.* 2008). High *H/L* ratios are associated in birds with susceptibility to infection (Al-Murrani *et al.* 2006), slow growth rates (Moreno *et al.* 2002) and survival to the next breeding season (Lobato *et al.* 2005, Kilgas *et al.* 2006b).

RBC values are related to body condition and are used as an indicator of health in birds (e.g. Kalmbach *et al.* 2004, Sergeant *et al.* 2004). RBC is an index of the oxygen transfer capacity of the blood. Low values are considered a sign of anaemia, because erythropoiesis (red blood cell production) depends on the nutritional state. It can also indicate illness or dehydration (Campbell 1995, Artacho *et al.* 2007). Individuals in good body condition have a greater number of RBCs than those in poor body condition (Sergeant *et al.* 2004); also, a reduction in body mass is associated with a decrease in RBC (Artacho *et al.* 2007).

Laboratory analyses

We divided the blood samples into subsamples in order to carry out particular analyses: 5 μ l to make a blood smear for the differential leukocyte counts and 5 μ l for the RBC counts. We stained air-dried blood smears using the May-Grünwald-Giemsa method with a Wescor "Aerospray Haematology" cytocentrifuge. To assess the proportion of different types of leukocytes (lymphocytes, heterophils, monocytes, eosinophils and basophils), we examined 100 leukocytes at 1000 \times magnification under oil immersion. Because of the very low numbers or absence of monocytes, basophils and eosinophils in most of the blood smears, we show and consider in the statistical analyses only the ratio of heterophils to lymphocytes. To determine the total red blood cell count (RBC), we counted the number of cells using a disposable, plastic counting chamber (Pentastar Slides, Medlab-Products, Raszyn, Poland), analogous to widely used Neubauer chamber. Before the count, we diluted the

blood 200 times and mixed it well with saline solution. The average time between sampling and the RBC count was 5 h. To obtain the final number of RBC cells ($\times 10^6$) in 1 μl of blood, we multiplied the sum of cells counted in the five squares by 90 (as the chamber volume, 0.011 μl , multiplied by 90 gives 1 μl) and the dilution (200 \times), and divided the product by the number of squares in which cells were counted.

Statistical analyses

To analyse the effects of categorical factors on different parameters we performed a GLM analysis with the *H/L* ratio, RBC and body mass as dependent variables, and sex, age and season as independent factors. Interaction terms were also included in the models. We carried out two separate analyses to investigate the effect of: (1) sex and season on the studied parameters in adults, and (2) sex and age on the studied parameters in individuals caught in summer (post-breeding season). Before the analyses, we normalized the *H/L* ratio using an arcsin square-root transformation (Zar 1999).

During the analysis of the effects of categorical factors on body mass (i.e. calculating body mass corrected for body size), we included wing length in the model as a covariate, to control for structural size differences (García-Berthou 2001). We chose wing length to represent the adult individual body size because of the significant correlation between this parameter and body mass in both immatures (both sexes combined; Pearson correlation coefficient, $r_{45} = 0.64$, $P < 0.0001$) and adults (both sexes combined; $r_{37} = 0.43$, $P = 0.008$). We compared fat scores among adult males and females caught in spring and summer, and also between individuals of different sex and age caught in summer using the non-parametric Kruskal-Wallis test. We performed all the statistical calculations using STATISTICA 8.0 (StatSoft Inc., Tulsa, Oklahoma).

Results

The body mass of adult reed buntings was affected significantly only by body size

(ANCOVA, wing length: $F_{1,32} = 4.99$, $P = 0.03$). Other factors, i.e. sex ($F_{1,32} = 0.24$, $P = 0.62$), season ($F_{1,32} = 0.07$, $P = 0.79$) and sex \times season interaction ($F_{1,32} = 0.63$, $P = 0.43$) did not affect body mass significantly. Thus, body mass corrected for body size (wing length) was similar in adult males [mean \pm SE (n): 20.0 \pm 0.45 g (21)] and females [mean \pm SE (n): 19.6 \pm 0.60 g (16)]. The fat scores of adult reed buntings were low and did not differ between sexes and seasons (median values for all categories: 0, quartiles 25%–75%: 0–1; Kruskal-Wallis test: $H_{3,48} = 7.18$, $P = 0.66$).

In adults, the *H/L* ratio was significantly affected by season ($F_{1,45} = 5.29$, $P = 0.03$). Neither sex ($F_{1,45} = 2.39$, $P = 0.13$) nor sex \times season interaction ($F_{1,45} = 0.41$, $P = 0.53$) affected the *H/L* ratio. RBC was affected only by season ($F_{1,42} = 4.65$, $P = 0.04$). Neither sex ($F_{1,42} = 0.02$, $P = 0.89$) nor sex \times season interaction ($F_{1,42} = 0.01$, $P = 0.92$) affected the RBC. The *H/L* ratio and RBC were significantly higher in spring than in summer (Fig. 1).

Body mass in reed buntings caught in summer was affected significantly only by body size (ANCOVA, wing length; $F_{1,39} = 9.43$, $P = 0.004$). Other factors, i.e. sex ($F_{1,39} = 0.36$, $P = 0.55$), age ($F_{1,39} = 0.16$, $P = 0.69$) and age \times sex interaction ($F_{1,39} = 0.04$, $P = 0.83$) did not affect body mass significantly. Thus, body mass corrected for body size (wing length) was similar in immature and adult males [mean \pm SE (n): 20.0 \pm 0.44 g (26)] and females [mean \pm SE (n): 19.5 \pm 0.59 g (18)]. In summer, fat scores of reed bunting were low and did not differ either between immatures and adults or between males and females (median values for all categories: 0, quartiles 25%–75%: 0–1; Kruskal-Wallis test: $H_{3,54} = 5.22$, $P = 0.16$).

In individuals caught in summer, the *H/L* ratio was significantly affected by age ($F_{1,47} = 4.33$, $P = 0.04$). Neither sex ($F_{1,47} = 0.00$, $P = 0.96$) nor sex \times age interaction ($F_{1,47} = 0.41$, $P = 0.52$) affected the *H/L* ratio. RBC was affected only by age ($F_{1,42} = 6.10$, $P = 0.02$). Neither sex ($F_{1,42} = 0.08$, $P = 0.78$) nor sex \times age interaction ($F_{1,42} = 0.06$, $P = 0.82$) affected RBC. In individuals caught in summer, the *H/L* ratio and RBC were higher in immatures as compared with those in adults (Fig. 2).

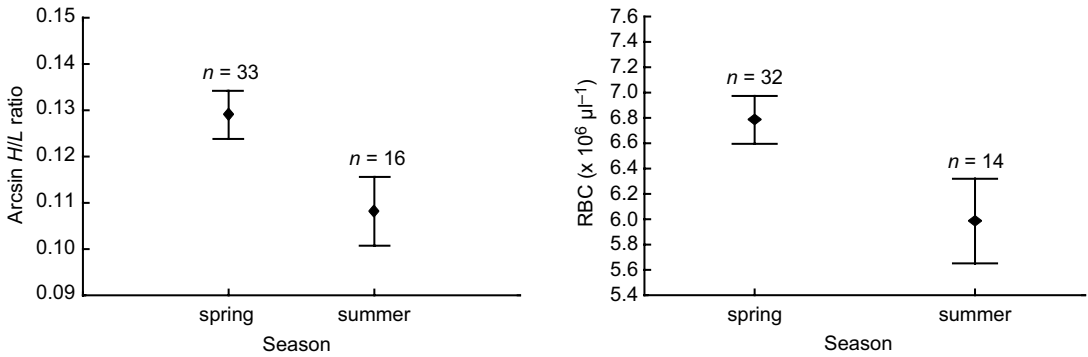


Fig. 1. Mean \pm SD of the H/L ratio (for arcsin square root transformed data) and RBC in adult reed buntings caught in spring and summer.

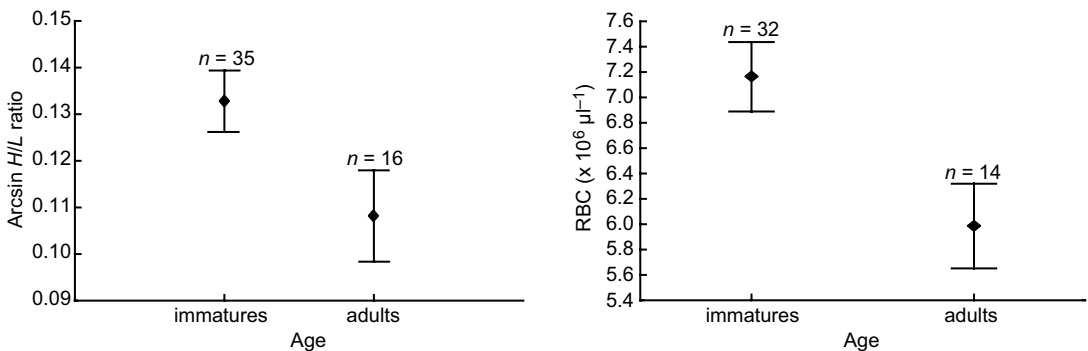


Fig. 2. Mean \pm SD of the H/L ratio (for arcsin square root transformed data) and RBC in immature and adult reed buntings caught in summer.

Discussion

The adult reed buntings that we caught had a higher H/L ratio in spring (breeding) than in summer (post-breeding). Elevated H/L ratios during breeding are also reported for other birds (e.g. Mallory *et al.* 2005, Norte *et al.* 2009) and may suggest the allocation of energy and nutrients from the immune function to reproduction, leading to physiological constraints and trade-offs (Norris & Evans 2000). Alternatively, an elevated H/L ratio may reflect physiological adjustment in response to expected increased demands associated with reproduction. According to the concept of allostasis (McEwen 2002, Schulkin 2003), the elevated glucocorticoid levels (resulting in an increase in the H/L ratio) observed in birds during breeding (Romero 2002) maintain internal systems within a heightened operating range to support expected reproduction demands. This elevation does not rep-

resent a reproduction stress response as it falls well below stress-related concentrations (Landys *et al.* 2006). Moreover, the adult reed buntings caught in our study during the post-breeding period were in the process of moulting. Lower numbers of heterophils and/or H/L ratios are reported for moulting birds (e.g. Driver 1981, Hawkey *et al.* 1989, Sanz *et al.* 2004), suggesting activation of the immune system. Indeed, in at least some bird species, an increase in the size of the immune system organs such as the thymus or spleen was observed during moult, which may be due to a need for heightened lymphocyte production associated with the expansion of the circulation or the need to avoid infection of wounds resulting from breakage of growing feather follicles (Ward & D'Cruz 1968, Silverin *et al.* 1999). Also, the glucocorticoid concentration in small passerines that undergo a complete moult after the breeding season is often lowest (Romero 2002), which is associated with a low H/L ratio.

Moreover, the low RBC values recorded in adult reed buntings caught during the post-breeding period are characteristic of moulting birds (Ghebremeskel *et al.* 1992, Hawkey *et al.* 1989, Sergeant *et al.* 2004).

In summer, *H/L* ratios in immature reed buntings were significantly higher than in adults, which may be indicative of a disease process (Davis *et al.* 2004, 2008). However, the high RBC values characteristic of individuals in good health and body condition (Sergeant *et al.* 2004) did not confirm this. The elevated *H/L* ratios in immatures may have resulted from the higher stress level in inexperienced, less efficiently foraging and/or socially lower-ranked immatures (Woodrey & Moore 1997). Similar age differences in the *H/L* ratio were observed in the herring gull (*Larus argentatus*) and feral pigeon (*Columba livia*) (Totzke *et al.* 1999, Pavlak *et al.* 2005), whereas the opposite pattern was found in other species of birds (e.g. Fairbrother & O'Loughlin 1990, Alonso *et al.* 1991, Quillfeldt *et al.* 2008, Norte *et al.* 2009). This discrepancy between the studies may be due to the variability of environmental (food abundance) and social (intensity of competition) conditions affecting stress levels. Alternatively, it may be due to the different time of sampling, since an increase in *H/L* with age, regardless of body condition, was found in some birds (e.g. Fairbrother & O'Loughlin 1990, Quillfeldt *et al.* 2008). We found that adult reed buntings had lower RBC values than immatures, which is in agreement with the results reported for the herring gull (Totzke *et al.* 1999) but contradicts the pattern observed in other bird species (Hawkey *et al.* 1989, Alonso *et al.* 1991, Gayathri *et al.* 2004, Pavlak *et al.* 2005). It is possible that the *H/L* ratios and RBCs recorded in adult reed buntings were influenced by the complete post-breeding moult undergone by the majority of individuals in this age category caught in summer, as the reported values of both parameters are characteristic of moulting birds (Driver 1981, Hawkey *et al.* 1989, Sergeant *et al.* 2004). In this context, the contradictory results of the previously mentioned studies may have been due to different moult strategies and/or sampling times.

Despite the differences in the levels of parental care provided by males and females in reed

buntings, their *H/L* ratios during and after breeding were not affected by sex. This result of our study was unexpected, in view of the fact that during breeding the *H/L* ratio in females of another passerine with biparental care [great tit (*Parus major*)] was higher than in males, possibly due to the greater contribution to offspring rearing and/or the high initial breeding investments in females (Hörak *et al.* 1998, Ots *et al.* 1998, Kilgas *et al.* 2006a, Krams *et al.* 2010). On the other hand, Norte *et al.* (2009) found no sex differences in *H/L* in the same species. This demonstrates that leukocyte profiles in breeding males and females may differ between populations and seasons according to local environmental conditions. Alternatively, it is possible that in the reed bunting, the higher initial breeding investments in females (egg production, incubation) were balanced by the males' outlay for pre-laying mate guarding and singing during the pre-laying and nesting periods (Cramp 1998, Bouwman *et al.* 2005, Marthinsen *et al.* 2005). Similarly, the lack of significant sex differences in body mass corrected for size and fat reserves suggests a similar protein content (e.g. muscle condition) in males and females, immatures and adults.

In conclusion, we found that seasonally dependent activities like breeding and moulting affected RBC and immune cell profiles in adult reed buntings. In contrast, their body condition was not affected by season. Adults and immatures caught during the post-breeding period differed in *H/L* ratio and RBC, but their body condition was similar. However, it is still not clear which physiological processes are responsible for the observed differences. This highlights the great need for comparative studies investigating changes in immune cell profiles in the annual cycle and physiological ones investigating the causal mechanism of those changes.

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