



Leukocyte profiles indicate nutritional, but not moulting stress in a migratory shorebird, the Common Snipe (*Gallinago gallinago*)

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Abstract

The relative ratio of the two main leukocyte types, heterophils (H) and lymphocytes (L), is known to change proportionally to the concentrations of stress hormones in the circulating blood. Thus, analysis of leukocyte profiles serves as a reliable proxy of stress in vertebrates, as high H/L ratios indicate stronger stress response. Moulting and migration are among the most energetically demanding and nutritionally stressful processes in the annual cycle of wild birds. Although most birds separate these two activities in time, a moulting–migration overlap has been reported for several avian species. The aim of this study was to examine whether overlapping energy requirements of moulting and migration produce an increased organismal stress in a shorebird species, the Common Snipe (*Gallinago gallinago*). For this purpose, we assessed leukocyte profiles and nutritional condition in more than 350 moulting and non-moulting snipe during autumn migration through central Europe. We found negative correlations between H/L ratios and different measures of condition, including size-corrected body mass, fat load, and plasma concentrations of triglycerides and total protein. However, no evidence was found for the effect of moulting status on H/L ratios. Our study indicates that while migration in a poor nutritional state can be associated with elevated stress response, the process of feather replacement may not constitute a serious stress for migrating Common Snipe. We suggest that these results may help to explain the occurrence of moulting–migration overlap in the Common Snipe and possibly in other avian species.

Keywords Common snipe · *Gallinago gallinago* · Heterophil/lymphocyte ratio · Migration · Moulting stress response

Zusammenfassung

Leukozytenprofile einer ziehenden Watvogelart, der Bekassine (*Gallinago gallinago*), sind ein Stress-Indikator für Nahrungsmangel, nicht jedoch für Mauser

Das Verhältnis der zwei wesentlichen Leukozytentypen, der Heterophilen (H) und der Lymphozyten (L), zueinander ändert sich bekannter Weise proportional zu Konzentrationen von Stresshormonen im Blutkreislauf. Daher dient die Analyse der Leukozytenprofile in Wirbeltieren als verlässlicher Stressindex, wobei höhere H/L Verhältnisse eine stärkere Stressantwort bedeuten. Im Jahreszyklus von Wildvögeln zählen Mauser und Zug zu den energieaufwändigsten und am ehesten Ernährungsstress verursachenden Prozessen. Wenngleich die meisten Vogelarten diese zwei Aktivitäten zeitlich trennen, wurde für mehrere Arten eine Überlappung von Mauser und Zug festgestellt. Ziel der vorliegenden Arbeit war die Untersuchung ob der sich überlagernde Energiebedarf von Mauser und Zug bei der Bekassine (*Gallinago gallinago*), einer Watvogelart, zu erhöhtem organismischen Stress führt. Dafür bestimmten wir die Leukozytenprofile und den Ernährungszustand von über 350 mausernden und nicht-mausernden Bekassinen auf ihrem Herbstzug durch Mitteleuropa. Wir fanden negative Korrelationen zwischen den H/L Verhältnissen und verschiedenen Maßen für die Körperkondition, inklusive größenkorrigierter Körpermasse, Fettanlagerung und Plasmakonzentrationen von Triglyceriden und Gesamtprotein. Wir fanden allerdings keinen Hinweis darauf, dass der Mauserstatus einen Effekt auf das H/L-Verhältnis hat. Während

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unser Ergebnis darauf hindeutet, dass Zugverhalten unter schwachen Ernährungsbedingungen mit erhöhter Stressantwort in Verbindung gebracht werden kann, scheint der Prozess des Gefiederwechsels für ziehende Bekassinen keinen wesentlichen Stress zu verursachen. Wir weisen darauf hin, dass unser Ergebnis helfen könnte, das Auftreten von Mauser-Zugüberlappung bei der Bekassine und möglicherweise auch bei anderen Vogelarten zu erklären.

Introduction

Moulting and migration are among the most energetically demanding and nutritionally stressful processes in the annual cycle of wild birds (e.g. Alerstam and Lindström 1990; Hemborg and Lundberg 1998). Nutritional costs of moult are primarily associated with increased amino acid metabolism necessary for renewal of feathers, as well as with increased heat loss caused by reduced insulation (Lindström et al. 1993; Klaassen 1995; Portugal et al. 2007). Feather production results in a deposition of proteins that constitute up to 25% of lean body mass (Murphy and Taruscio 1995), and this process is associated with substantial increase of metabolic rate (Croxall 1982; Guillemette et al. 2007). Also, flight-feather moult may impose additional energetic costs resulting from reduced flight performance (Swaddle and Witter 1997). This is especially important for migrating birds, as long-distance flights require large energy expenditure, even if wings are not aerodynamically impaired (Wikelski et al. 2003). Finally, tight temporal constraints of migration usually limit the time available for feeding activities, which can exacerbate nutritional stress in migrants (Weber and Houston 1997).

Since an overlap between moult and migration can produce a serious trade-off in resource allocation, these two activities are usually separated in time (Payne 1972). However, several bird species have been reported to moult during migration, although the magnitude of this overlap may differ between taxa (Hall and Fransson 2001; Pérez-Tris et al. 2001; Remisiewicz 2011). An extensive moult–migration overlap has been described, among other species, in the Common Snipe (*Gallinago gallinago*). The Common Snipe is a medium-sized shorebird that breeds across northern Eurasia (Snow and Perrins 1998), and individuals from the Western Palaearctic breeding range winter mostly in southwestern Europe (Minias et al. 2010a). The species uses a strategy of energy minimization during autumn migration, meaning that birds accumulate relatively small fat reserves and frequently stop over en route (Włodarczyk et al. 2007). Consequently, the overall pace of migration is slow, which imposes serious time constraints in the annual cycle, often resulting in a moult–migration overlap (Włodarczyk et al. 2008). As a result, most young snipe start the postjuvenile moult in the middle of the autumn migration, whereas adults start the postbreeding moult as soon as reproductive

activities have concluded, before departure on migration (Włodarczyk et al. 2008; Podlaszczuk et al. 2016). As in many other bird species (e.g. Klaassen 1995; Guillemette et al. 2007), the process of moult in the Common Snipe has been shown to be energetically costly. Young snipe were found to have lost more than 50% of their fat reserves during the postjuvenile moult (Minias et al. 2010b), and depletion of endogenous fat was accompanied by a significant decrease in plasma concentrations of triglycerides during the advanced stage of moult (Podlaszczuk et al. 2017). However, it remains unknown whether overlapping costs of moult and migration produce elevated levels of organismal stress in species such as the Common Snipe.

Although incorporating stress into ecological and eco-physiological research has become increasingly popular within the last few decades (Wingfield et al. 1997), measurements of adrenal stress hormones (usually corticosterone) have certain constraints that deter ecologists from their wide application in the field (Romero 2004). Thus, many researchers assess leukocyte profiles, which are widely acknowledged as a reliable proxy of physiological stress in vertebrates (Davis et al. 2008). Leukocyte profile is the relative proportion of white blood cell types, two of which (lymphocytes and heterophils/neutrophils) readily respond to organismal stress (Maxwell 1993). Lymphocytes mediate antigen-specific responses, whereas heterophils (equivalent of neutrophils in birds and reptiles) show high phagocytic activity towards a wide spectrum of pathogens and they are especially active in inflammatory lesions (Harmon 1998). A stressful environment depresses the number of lymphocytes in peripheral circulating blood and induces an influx of heterophils from bone marrow (so-called leukocyte trafficking), which reflects heightened innate immunity in preparation for injury and resulting infectious challenges (Johnstone et al. 2012). The level of these changes is proportional to the concentrations of stress hormones in the blood (Van Dijk et al. 1979). Consequently, the ratio of heterophils to lymphocytes (H/L ratio) is expected to indicate an overall stress level, where high ratios are associated with stronger stress response (Gross and Siegel 1983; Ruiz et al. 2002; Davis et al. 2004).

The aim of this study was to examine whether overlapping nutritional requirements of moult and migration produce increased organismal stress in the Common Snipe. For this purpose, we tested two alternative hypotheses: (1) moulting during migration increases stress response of birds

because of large nutritional costs of feather growth and/or increased predation risk associated with reduced flight performance of moulting birds (H/L ratios higher in moulting than non-moulting individuals) and (2) stress response of birds during migration is associated with their nutritional state, irrespective of moult (H/L ratios do not vary with moult, but negatively correlate with nutritional condition). To test these hypotheses we assessed leukocyte profiles and different proxies of nutritional condition (size-corrected body mass, fat load, plasma concentrations of triglycerides and total protein) in more than 350 moulting and non-moulting Common Snipe captured during autumn migration through central Europe.

Methods

General field procedures

The data were collected during autumn migration of the Common Snipe at Jeziorsko reservoir (51°40'N, 18°40'E), central Poland. Common Snipe were captured in walk-in traps. Fieldwork was conducted in 2009–2015 between 23 July and 25 September. In total, 1292 Common Snipe were captured. Each bird was ringed and aged by plumage (Kaczmarek et al. 2007; Włodarczyk et al. 2008). Sex determination was based on species-specific discriminant equations (Włodarczyk et al. 2011). For this purpose, bill length and the distance between the tips of two neighbouring outermost tail feathers were measured with callipers (± 0.1 mm), whereas vane length of the outermost tail feather was measured with a ruler (± 1 mm). The following morphological measurements were also collected: wing length (± 1 mm), head length (± 0.1 mm), and tarsus length (± 0.1 mm). All birds were weighed on an electronic balance (± 1 g). Fat load was visually assessed in the furculum and axilla with use of a five-point scale developed for waders (Meissner 2009). The two measures were averaged within each individual and used as one of the proxies for body condition.

As both adult and juvenile snipe can actively moult during autumn migration (Włodarczyk et al. 2008), moult status of each individual was recorded. Juvenile (first-year) birds were classified into one of three moult categories: (1) premoult stage (no feathers moulted); (2) initial stage of moult (only body feathers and wing coverts in active moult); (3) advanced stage of moult (tertials or tail feathers in active moult). We could not collect data for first-year snipe that completed postjuvenile moult, as such birds attain full adult-like plumage and cannot be distinguished from adults on the basis of plumage characteristics. In contrast, adults often retain one to several unmoulted

humeral coverts after moult, which can be used as a key characteristic to identify adults that have completed postbreeding moult (Kaczmarek et al. 2007). Thus, adult snipe were classified as either in (1) the advanced stage of moult (flight feathers in active moult) or (2) the postmoult stage (all flight feathers fully grown). Data could not be collected for adult snipe in the premoult stage because virtually all adults start moulting before departure on migration.

Leukocyte profiles

Of all captured snipe, we randomly selected 374 individuals (28.8%; 283 young birds and 91 adults) to prepare blood smears. Selected birds represented different moult categories, and the following sample sizes were obtained for each moult stage: 127 for premoult stage, 82 for initial moult stage, and 74 for advanced moult stage for young birds; 30 for advanced moult stage and 61 for postmoult stage for adults. Differences in sample sizes between moult stages reflected the true distribution of moult categories in our migratory population of snipe. The handling time of sampled birds (including transport from walk-in traps) was less than 1 h and in most cases less than 45 min. Although it has been reported that avian heterophil or lymphocyte counts can occasionally change within 60 min of capture (Cīrule et al. 2012), studies on shorebirds (Buehler et al. 2008; D'Amico et al. 2017) and some passerines (Davis 2005) indicated that H/L ratio is relatively stable within the first hour after capture. Consequently, we assumed that the measurements of shorebird leukocyte profiles were unlikely to be affected by handling stress under our sampling protocol. The ulnar vein of each bird was punctured with a disposable needle, and a drop of blood was transferred to a slide and smeared. Blood smears were air-dried and stored in darkness until analysis. All smears were stained by the May–Grünwald–Giemsa method and scanned at $\times 1000$ magnification under a light microscope. A sample of 100 leukocytes from each blood smear was differentiated into five cell types: heterophils, lymphocytes, eosinophils, basophils, and monocytes. The H/L ratio was calculated by division of the number of heterophils by the number of lymphocytes. To reduce variability, all blood smears were assessed by one of the authors (RW). Within-individual repeatability of H/L ratio calculations measured with the intraclass correlation coefficient (Lessells and Boag 1987) was high ($R = 0.86$, $n = 25$, $P < 0.001$). The distribution of H/L ratio was highly right skewed (skewness 3.69), and thus all ratios were log-transformed to obtain a distribution reasonably close to normal (skewness -0.23).

Basic condition indices

We used body mass adjusted for structural size as the second body condition index. To derive this estimate we used the scaled mass index recommended by Peig and Green (2009), which was calculated according to the following equation:

$$\hat{M}_i = M_i \left[\frac{L_0}{L_i} \right]^{b_{\text{SMA}}}$$

where M_i and L_i are the body mass and the linear body measurement of individual i , respectively, b_{SMA} is the scaling exponent estimated by the standardized major axis regression of body mass on linear body measurement, L_0 is the mean value of the linear body measurement for the study population, and \hat{M}_i is the predicted body mass for individual i when the linear body measure is standardized to L_0 . We used tarsus length (mean \pm standard error 33.98 ± 0.06 mm) as a linear body measurement, since it showed the strongest correlation with body mass on a log–log scale among all biometrical traits collected ($r = 0.30$, $n = 366$, $P < 0.001$), indicating that it best explained the fraction of mass associated with structural size (Peig and Green 2009). b_{SMA} was estimated with the software program RMA (Bohonak 2004) with 100,000 bootstraps (0.3877 ± 0.0194).

Plasma protein and triglycerides

We measured plasma concentrations of triglycerides and total protein as additional indices of physiological condition in the Common Snipe. In general, plasma chemistry is regarded as a valuable tool to assess condition in wild-living birds (Jenni-Eiermann and Jenni 1998). Specifically, plasma concentrations of triglycerides and total protein have been shown to correlate positively with food intake rate in several avian species (e.g. Totzke et al. 1999; Quillfeldt et al. 2004). Low levels of total proteins and triglycerides are often characteristic of short periods of food deprivation and covary with size-corrected body mass of birds (Boismenu et al. 1992; Dawson and Bortolotti 1997; Guglielmo et al. 2005). Also, plasma triglyceride concentrations have been widely used as indicators of mass gain and refuelling performance in shorebirds (Williams et al. 1999; Seaman et al. 2005).

Plasma triglycerides and total protein were measured in 305 birds and 359 birds, respectively. Although plasma chemistry in shorebirds has been reported to change within 45 min of capture (Williams et al. 2007), we were not able to sample snipe for blood much more quickly, since transport of birds from walk-in-traps to the ringing station took approximately 30 min. After arrival at the ringing station, birds were immediately bled. However, we took utmost care to keep our catching and sampling protocols constant throughout the entire study, and thus interindividual variation in

handling time was deemed minimal and expected not to bias the results of our plasma analyses. This approach followed previous research on plasma chemistry in shorebirds (e.g. Lyons et al. 2008).

We collected 20–40 μL of blood from the ulnar vein of each bird in heparinized capillary tubes. Samples were centrifuged at 6000 rpm for 5 min within 1 h of collection to separate plasma from blood cells, and were kept at -20°C until analysis. In 2009–2012, plasma concentrations of total protein ($n = 165$) were measured with a calibrated temperature-compensated veterinary refractometer (Vet 360 TS meter, Reichert Analytical Instruments, Depew, NY, USA). Plasma total protein concentrations for the remaining samples ($n = 194$; 2013–2015) and plasma triglyceride concentrations ($n = 305$; 2012–2015) were measured with a spectrophotometer (BTS-330, BioSystems Reagents and Instruments, Barcelona, Spain) with use of commercial kits and reagents from the same manufacturer (BioSystems Reagents and Instruments, Barcelona, Spain). All spectrophotometric analyses were conducted according to the manufacturer's protocols with a biuret reaction for the measurement of total protein and glycerol phosphate oxidase/peroxidase for the measurement of triglycerides. The absorbance of each sample was measured in a flow cuvette against a blank reagent at the following wavelengths: 545 nm (total protein) or 500 nm (triglycerides). Since the two measurement methods yielded significant differences in plasma total protein estimates ($F_{1357} = 93.54$, $P < 0.001$), we removed this variation by extracting residuals from the ANOVA model, which were subsequently used as a measure of plasma total protein concentration in the analyses. The distribution of plasma triglyceride concentrations was reasonably close to normal (skewness 0.75), and thus raw data were entered in the analyses.

Statistical analyses

The effects of age and sex on H/L ratio were initially tested by factorial ANOVA design which included an interaction between the two predictors. Relationships of H/L ratio with different condition indices and moult stage were assessed with general linear models. Each index was entered as a covariate in a separate general linear model to avoid multicollinearity of predictor variables. Time of day (hour) and Julian date were included in each model as covariates to control for diurnal and intraseasonal variation in physiological state and condition. The effects of age, year, and moult stage were included as fixed factors. Moult stage was nested within age because different moult categories were distinguished for young and adult birds. We also included age–condition and moult–condition interactions to test whether the relationships between H/L ratio and condition were similar across age classes and moult stages. To obtain more parsimonious

Table 1 Means \pm standard error (SE) for condition indices and ratio of heterophils to lymphocytes (H/L ratio) in young ($n = 282$) and adult ($n = 91$) Common Snipe migrating through central Poland

Factor	Young birds		Adult birds	
	Mean	SE	Mean	SE
Scaled mass index (g)	99.01	0.55	98.55	0.90
Fat score	0.78	0.05	0.90	0.08
Plasma triglycerides (mg/dL)	78.44	2.13	75.59	3.47
Total plasma protein (g/L)	34.50	0.43	34.71	0.85
H/L ratio	3.45	0.13	4.82	0.29

Because of the log-normal distribution, geometric mean \pm SE is shown for the H/L ratio.

reduced models we removed non-significant ($P > 0.05$) predictors from the initial full models. All models were run in JMP 13.0 (SAS Institute, Cary, NC, USA). Effect sizes were estimated with the partial eta squared, as implemented in Statistica 12 (StatSoft, Tulsa, OK, USA). All values are presented as the mean \pm standard error.

Results

We found significant age variation in H/L ratios of the Common Snipe ($F_{3341} = 9.66$, $P = 0.002$), as adults had higher H/L than young birds (Table 1). The effect of age was similar for both sexes, as indicated by non-significant age–sex interaction ($F_{1341} = 0.01$, $P = 0.92$). The main effect of sex on H/L ratio was also non-significant ($F_{1341} = 0.02$, $P = 0.87$).

We found that H/L ratio was significantly related to all measures of nutritional condition, including size-corrected body mass, fat load, and plasma concentrations of triglycerides and total protein (Tables 2, 3). All these relationships were negative (Fig. 1), indicating that individuals in a poor nutritional state showed higher levels of physiological stress. Despite statistical significance of these relationships, the effect sizes for each condition index were low (partial eta squared 0.013–0.053). In contrast to nutritional condition, we found no evidence for an effect of moult stage on H/L ratio in the Common Snipe (Tables 2, 3). Interactions of condition indices with age and moult were non-significant in all the models (Tables 2, 3), indicating that the relationships of H/L ratio and condition were similar across age classes and different moult stages. No support was found for diurnal or intraseasonal variation in H/L ratios (Tables 2, 3).

Discussion

Our study supports the hypothesis that poor physiological condition during migration may increase the level of

Table 2 Models assessing relationships of basic condition indices, scaled mass index (SMI), and fat score (FS), with ratio of heterophils to lymphocytes in the Common Snipe

Predictors	<i>F</i>	<i>P</i>	$\beta \pm$ SE
Full model (SMI)			
SMI	<i>10.68</i>	<i>0.001</i>	-0.021 ± 0.007
Age	<i>17.29</i>	<i>< 0.001</i>	0.22 ± 0.05
SMI \times age	0.11	0.74	-0.002 ± 0.006
Time of day	2.72	0.10	-0.011 ± 0.006
Date	1.01	0.32	-0.005 ± 0.005
Moult stage	0.65	0.59	–
SMI \times moult stage	1.23	0.30	–
Year	<i>11.63</i>	<i>< 0.001</i>	–
Reduced model (SMI)			
SMI	<i>13.01</i>	<i>< 0.001</i>	-0.021 ± 0.005
Age	<i>19.59</i>	<i>< 0.001</i>	0.21 ± 0.05
Year	<i>20.12</i>	<i>< 0.001</i>	–
Full model (FS)			
FS	<i>6.13</i>	<i>0.014</i>	-0.18 ± 0.07
Age	<i>22.75</i>	<i>< 0.001</i>	0.26 ± 0.05
FS \times age	0.88	0.35	-0.065 ± 0.070
Time of day	2.86	0.092	-0.011 ± 0.007
Date	0.72	0.40	-0.004 ± 0.005
Moult stage	0.33	0.80	–
FS \times moult stage	0.77	0.51	–
Year	<i>9.66</i>	<i>< 0.001</i>	–
Reduced model (FS)			
FS	<i>7.64</i>	<i>0.006</i>	-0.15 ± 0.05
Age	<i>22.56</i>	<i>< 0.001</i>	0.23 ± 0.05
Year	<i>11.95</i>	<i>< 0.001</i>	–

The sample sizes are 366 and 373 individuals for SMI and FS, respectively. Significant predictors are marked in italic. Estimates [$\beta \pm$ standard error (SE)] are presented for all predictors except for multilevel fixed factors (year, moult stage) and their interactions.

organismal stress, as measured with H/L ratios. We found that all four indicators of condition, including fat load and plasma metabolite concentrations, negatively correlated with H/L ratios of the Common Snipe, indicating an elevated stress response of birds migrating with low energy reserves. In contrast, we found no evidence for the effect of moult status on leukocyte profiles, suggesting that despite its expected energetic burden, the process of feather replacement may not constitute a serious physiological stress for migrating Common Snipe.

Although we are aware of no previous research that examined relationships between plasma metabolite concentrations and H/L ratios in migrating birds, many studies have reported elevated H/L ratios in individuals with low body mass or experiencing food deprivation (Ots et al. 2001; Gladbach et al. 2010; Krams et al. 2011; Jakubas et al. 2015; but see Krams et al. 2012). For example, male Upland Geese (*Chloephaga picta leucoptera*) showed negative correlation

Table 3 Models assessing relationships of plasma concentrations of triglycerides (TGL) and total protein (TP) with ratio of heterophils to lymphocytes in the Common Snipe

Predictors	<i>F</i>	<i>P</i>	$\beta \pm \text{SE}$
Full model (TGL)			
TGL	<i>4.79</i>	<i>0.029</i>	-0.005 ± 0.002
Age	<i>15.15</i>	<i>< 0.001</i>	0.23 ± 0.06
TGL \times age	0.02	0.88	-0.0003 ± 0.0020
Time of day	2.91	0.089	-0.011 ± 0.007
Date	0.23	0.63	-0.003 ± 0.005
Moult stage	0.64	0.59	–
TGL \times moult stage	0.40	0.75	–
Year	3.78	<i>0.003</i>	–
Reduced model (TGL)			
TGL	<i>9.64</i>	<i>0.002</i>	-0.005 ± 0.002
Age	<i>18.37</i>	<i>< 0.001</i>	0.22 ± 0.05
Year	<i>4.02</i>	<i>0.001</i>	–
Full model (TP)			
TP	<i>6.20</i>	<i>0.013</i>	-0.019 ± 0.008
Age	<i>20.65</i>	<i>< 0.001</i>	0.25 ± 0.05
TP \times age	3.44	0.064	-0.014 ± 0.008
Time of day	0.47	0.49	-0.005 ± 0.007
Date	0.97	0.33	-0.005 ± 0.005
Moult stage	0.44	0.73	–
TP \times moult stage	0.61	0.61	–
Year	7.59	<i>< 0.001</i>	–
Reduced model (TP)			
TP	<i>4.60</i>	<i>0.033</i>	-0.014 ± 0.006
Age	<i>19.81</i>	<i>< 0.001</i>	0.22 ± 0.05
Year	<i>12.42</i>	<i>< 0.001</i>	–

The sample sizes are 305 and 359 individuals for TGL and TP, respectively. Significant predictors are marked in italic. Estimates [$\beta \pm$ standard error (SE)] are presented for all predictors except for multilevel fixed factors (year, moult stage) and their interactions.

between H/L ratio and body mass adjusted for structural size (Gladbach et al. 2010), whereas subordinate individuals of the Great Tit (*Parus major*) had the highest decrease in body mass and the highest increase in H/L ratio during cold spells in winter (Krams et al. 2011). In contrast, lack of correlation between H/L ratio and size-corrected body mass or fat load has been reported for migrating *Catharus* and *Hylocichla* thrushes (Owen and Moore 2006). However, most research suggest that unfavourable feeding conditions can provoke changes in leukocyte profiles of wild birds. Elevated H/L ratios have been recorded in passerine nestlings raised in poor-quality territories with low availability of food resources (Soursa et al. 2004; Bańbura et al. 2013). A similar relationship was found in juvenile Common Kestrels (*Falco tinnunculus*) (Müller et al. 2011). Adult Little Auks (*Alle alle*) had higher H/L ratios during breeding seasons with unfavourable feeding conditions than in seasons with

abundant food supply (Wojczulanis-Jakubas et al. 2011). In many avian species, migration can be associated with periods of short-term fasting resulting from long-distance flights and necessity to find appropriate stopover sites (Alerstam and Lindström 1990). Also, environmental conditions at stopover sites are often unpredictable and can show large annual variation (Russell et al. 1994). Although some birds can possibly use their previous experience to make stopover decisions (Minias et al. 2010c), general unpredictability of food resources along the flyway may have a detrimental effect on physiological and nutritional status of migrants, resulting in an elevated stress response in those that are not able to meet the high energetic demands of migration (Owen and Moore 2006). This can be especially important for the Common Snipe, which migrates with small fat reserves and relies on numerous stopover sites along its migratory route (Włodarczyk et al. 2007). Our results suggest that a combination of low energy reserves and insufficient food availability at stopover sites can produce stressful conditions and elevated H/L ratios, at least in some migrating snipe.

Despite a general correlative link between stress and nutritional status of migrating snipe, we found no evidence for an effect of moult on H/L ratios. Although leukocyte profiles have been reported to correlate with moult in other wild birds, the explanation for this association is far from straightforward. For example, juvenile White Wagtails (*Motacilla alba*) with a larger extent of partial postjuvenile moult showed higher H/L ratios than individuals with a lower number of moulted feathers (López et al. 2005). This relationship was suggested to be primarily mediated by intraspecific social interactions, as juveniles with more adult-like plumage experienced more aggressive behaviour from adult conspecifics, which could increase their levels of stress (López et al. 2005). In several other passerine species, H/L ratios were lower in moulting individuals than in non-moulting individuals, supporting the hypothesis that the process of feather replacement does not cause a serious stress in birds (Sanz et al. 2004; Jakubas et al. 2011; Kulaszewicz et al. 2015). This pattern can be attributed to a few non-exclusive mechanisms. First, large energy reserves are required for initiation of the moulting process (Borras et al. 2004), and individuals that are in a poor nutritional state may postpone or suspend moult (Barta et al. 2008). This means that individuals that decide to moult earlier in the annual cycle are often in a relatively good condition and under low physiological stress, so even assuming that moult is stressful, its negative effect might be counterbalanced by the favourable physiological status of birds at the moment of moult initiation. Second, the immune system is activated at the beginning of moult, which is reflected by increasing size of immune organs, such as the thymus or spleen, as well as by elevated production of lymphocytes (Anderson 1970; Brake et al.

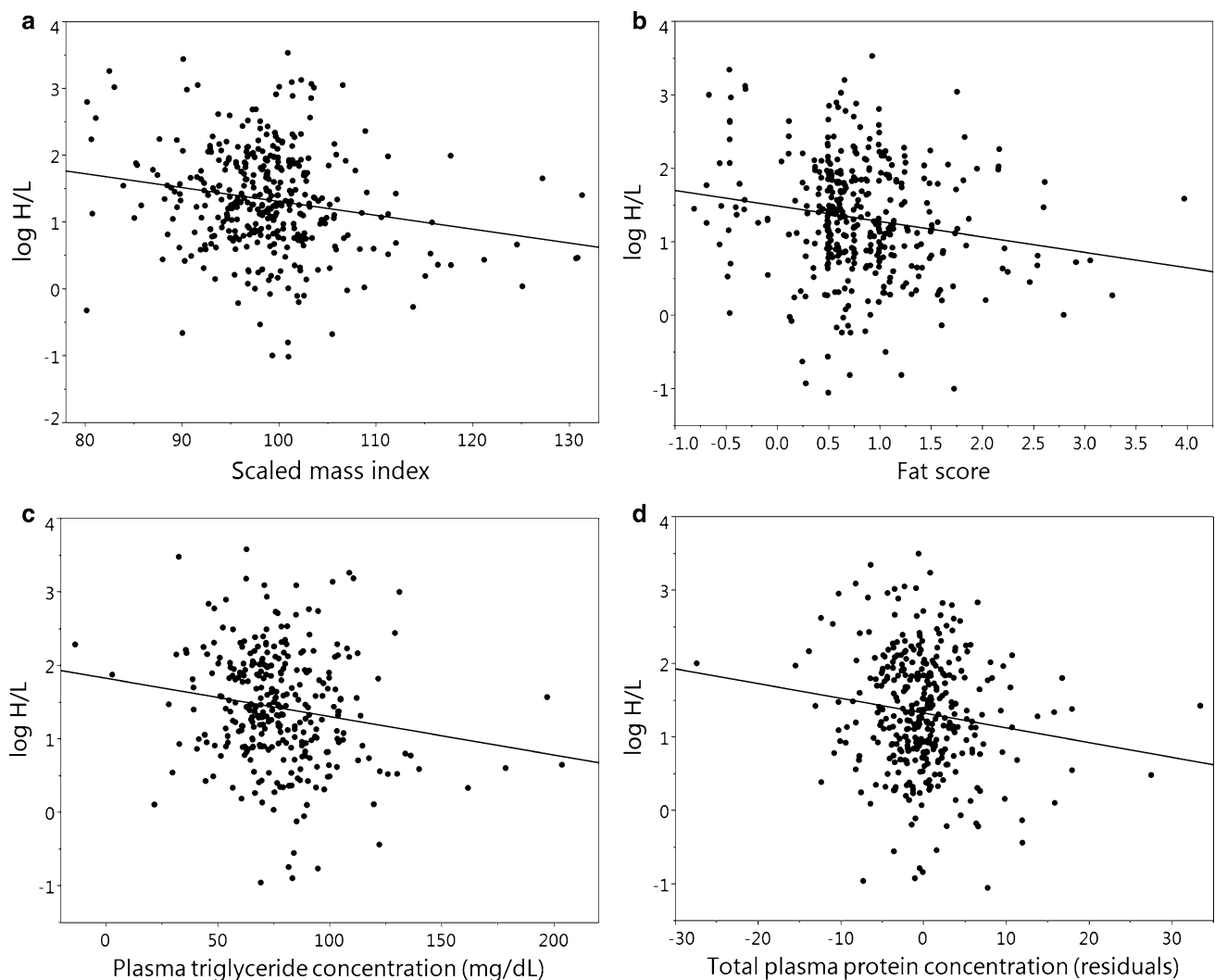


Fig. 1 Partial regression (effect leverage) plots for the relationships between ratio of heterophils to lymphocytes (H/L) and different condition indices in the Common Snipe (all adult and young birds com-

pared): scaled mass index (**a**), fat score (**b**), plasma triglyceride concentration (**c**), and plasma total protein concentration (**d**)

1981; Silverin et al. 1999). An increasing number of lymphocytes in circulating blood reduces H/L ratio, which is also accompanied by low concentrations of glucocorticoids in blood (Romero 2002). Third, corticosterone increases protein mobilization (i.e. breakdown), which may interfere with elevated protein requirements during moult (Murphy and King 1992; Romero et al. 1998). This is in agreement with the results of our study, indicating no significant variation in H/L ratios between successive stages of moult, as well as between moulting and non-moulting Common Snipe.

In conclusion, our study indicates that different energetically demanding processes within the annual life cycle of birds may entail differing stress response. Although general poor nutritional status during migration increased the level

of stress in the Common Snipe, stress response was unaffected by the process of moult. The latter suggests that stress response of birds may be fine-tuned to some other physiological processes, such as investment in immune response, and it may explain the occurrence of moult–migration overlap in the Common Snipe and possibly in other avian species.

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Author contributions RW and PM designed the study. RW, KK, TJ, and PM collected the data. RW and PP performed laboratory analyses. PM performed statistical analyses. RW, PP, KK, TJ, and PM wrote the article/revised the article for intellectual content.

Compliance with ethical standards

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