Journal of Avian Biology 47: 001–009, 2016
doi: 10.1111/jav.01000

Subject Editor: Ronald Ydenberg. Editor-in-Chief: Jan-Åke Nilsson. Accepted 28 June 2016

Trade-offs between reproduction and self-maintenance (immune function and body mass) in a small seabird, the little auk

Izabela Kulaszewicz, Katarzyna Wojczulanis-Jakubas and Dariusz Jakubas

I. Kulaszewicz (bioik@univ.gda.pl), K. Wojczulanis-Jakubas and D. Jakubas (http://orcid.org/0000-0002-1879-4342), Univ. of Gdańsk, Dept of Vertebrate Ecology and Zoology, Gdańsk, Poland.

Breeding season is the most energetically and physiologically demanding phase in the avian annual cycle, challenging adults' physiology and survival. However, the timing and extent that self-maintenance of breeding adults is compromised during the breeding season is poorly understood. We investigated the trade-off between reproduction and self-maintenance in relation to breeding phase (prelaying, incubation, chick rearing) and sex in a small Arctic seabird, the little auk *Alle alle*. To measure a bird's allocation of time for self-maintenance, we examined size-adjusted body mass and immunocompetence expressed by bacteria (*Escherichia coli*) killing capacity (BKC) of blood plasma, heterophils/lymphocyte ratio (H/L) and their numbers of particular leucocytes per 10 000 red blood cells (RBC). We found that size-adjusted body mass decreased as the breeding season progressed. BKC, number of heterophils and H/L values were all was significantly higher at prelaying when compared to all other phases. Interestingly, we found that heavier individuals had higher BKC and number heterophils at the prelaying and chick rearing phases than light individuals. There were no differences by sex in any studied variables. Our results indicate that immunocompetence and body mass of breeding adults decreases over the course of breeding season. The efficiency of the immune system appears to be dependent on the bird's body reserves. Our results suggest that little auks allocation of resources into reproduction negatively affects their self-maintenance.

The breeding season is one of the most time consuming and energetically demanding phases of a bird's annual cycle (Drent and Daan 1980, Walsberg 1983). During breeding, adult birds need more energy than in any other part of the year to meet the requirements associated with reproductive activities, such as courtship, territory and mate guarding, nest building, egg formation, egg incubation, and brooding and feeding the offspring (Martin 1987, Sandberg and Moore 1996). These energy needed to meet these requirements may be met by increased foraging intensity and reorganization of the time budget. However, a growing number of studies shows that breeders often fail to modify their foraging behaviour and time budget in a way that allows all of these extra energy expenditures to be met (Sol et al. 2002, Dehnhard et al. 2011, Dehnhard and Hennicke 2013, Jaeger et al. 2014, Powell et al. 2015). Moreover, according to the reproduction-immune system trade-off hypothesis (Deerenberg et al. 1997, Hasselquist and Sherman 2001, Martin et al. 2008), when the cost related to reproduction is at a maximum, the immune system is suppressed or down-regulated (Hasselquist and Sherman 2001) and immunity suppression during energetically taxing reproductive phases has been reported in some species (Adamo et al. 2001).

Body mass also changes due to various energy demands associated with reproduction, usually decreasing with increased effort. Sometimes these changes may be adaptive – the body mass loss after hatching increases mobility of the parent (Ankney and Macinnes 1978). Because interpretation of body mass changes during the breeding season is complicated, it should be only as an informative background for interpretation of changes in other variables.

The purpose of this study was to investigate the trade-off between reproduction and immune function and body mass in the little auk (or dovekie *Alle alle*). The little auk is a small High Arctic colonially breeding seabird. Its mass-specific daily energy expenditure is one of the highest among seabirds (Gabrielsen et al. 1991). Breeding adults invest heavily into the reproduction, starting from with a prolonged prelaying phase, when the female produces a large egg (19.2% of body mass) and the male guards his partner and nest site (Stempniewicz 2001, Wojczulanis-Jakubas and Jakubas 2012, Wojczulanis-Jakubas et al. 2014). This phase is followed with incubation and chick rearing which last one month each. Both partners are equally involved in parental duties (Harding et al. 2004, Wojczulanis-Jakubas and Jakubas 2012) during these phases. At the end of the chick rearing phase, females abandon the brood and the male stays with the offspring during fledging (Harding et al. 2004, Wojczulanis-Jakubas and Jakubas 2012). One hypothesis to explain this female brood desertion is that it is the result of her lowered body reserves (Wojczulanis-Jakubas and Jakubas 2012, Wojczulanis-Jakubas et al. 2012). Due to

these life-history traits and high energy demands, the little auk may serve as a good model species to investigate resource allocation in adults during the breeding, including sex differences.

In the light of reasoning presented above, we hypothesized that the immunocompetence, expressed as bacteria killing capacity (BKC), heterophils to lymphocytes (H/L) ratio, and the number of leucocytes per 10 000 RBC along with the size-adjusted body mass of parental birds would decrease with increasing parental investment over the course of the breeding season. We also expected that breeders with higher size-adjusted body mass (i.e. with larger body reserves) would have better immune function (i.e. higher BKC, lower H/L ratio, higher number of leucocytes per 10 000 RBC). Due to the female's investment in egg production, we expected them to have lower immunocompetence and size-adjusted body mass than males.

Methods

Study area and field work

This study was undertaken in Hornsund, SW Spitsbergen (77°00′N, 15°33′E) in 2014 during the following phases of the breeding season: a) prelaying, 20–21 d before the median of egg laying, b) incubation, 2–3 d after egg laying, c) incubation, 1–2 d before hatching, and d) day at 14–15 d during of the chick rearing. During the prelaying phase every adult bird was classified as a potential breeder. Individuals were assigned an active breeder status if their nest contained eggs or if during the incubation and chick rearing there was food in the gular pouch which was confirmed during the banding process. We captured the birds with noose-carpets deployed in the colony area during prelaying and chick rearing, or by hand, directly from the nest during the incubation phase. If we did not know the exact date of egg laying or hatching for individual birds, we assessed it based on the hatching median for that colony patch. The assessment error was not high considering highly synchronized hatching (Stempniewicz 1995, Jakubas and Wojczulanis-Jakubas 2013). We released birds or returned them directly to the nest unharmed after ca 10 min of handling.

We measured the flattened wing with a 1 mm accuracy ruler and the head to bill length and bill length with calliper of 0.01 mm accuracy. We then weighed each captured individual with an electronic balance of 0.1 g accuracy. From each bird, we collected a blood sample (ca 80 µl) by pricking the brachial vein and using a 100 µl heparinized capillary tube. All birds were ringed to prevent multi-sampling of the same individual. We kept the blood cool (+ 4°C) for 2–3 h until it could be divided into two subsamples: 1) ca 5 µl for leucocytes counts in a blood smear; 2) the remaining blood was centrifuged for 10 min at 6000 rpm to obtain a BKC (plasma) sample, and for molecular sexing (red cells). We kept the plasma cool for 1–2 d (+ 4°C) before the initiation of the laboratory procedure. Red cells were frozen at –20°C and analyzed within 4 months.

In total, we sampled 120 breeders. Fifteen males and 10 females were sampled during prelaying. Fifteen males and 16 females were sampled at the beginning of incubation. Twenty males and 17 females were sampled at the end of incubation, and 12 males and 15 females were sampled during the chick rearing phase). All individuals were sampled only once.

Study variables

To investigate changes in immune defence of adults at the consecutive phases of the breeding season, we measured their BKC and leucocyte profiles. BKC of bird's plasma encompasses multiple components of the innate immune system, including natural antibodies, complement proteins, and lysozyme (Matson et al. 2006) and is considered a measure of innate immunity (Merchant et al. 2005). A number of soluble proteins in the blood plasma also limit infections. Natural antibodies serve as non-specific recognition molecules with the ability to limit early microbial infection (Ochsenbein et al. 1999). To measure BKC of the little auk, we selected *Escherichia coli* because this is a ubiquitous bacteria, which should minimize the potentially confounding effect of different antigen exposure histories (Millet et al. 2007).

For the leucocyte profiles, we measured the relative numbers by percentage of different leukocyte types in the peripheral blood for the calculation of heterophils to lymphocytes (H/L) ratio as well as numbers of leucocytes per 10 000 erythrocytes. Both parameters provide a convenient measure of integrated immune function (Davis 2005, Salvante 2006). Elevated total number of leucocytes indicates an inflammatory process in response to both microbiological and macroparasite infections (Dein 1986). Lymphocytes, as a part of acquired immunity, are responsible for pathogen-specific immune response (Dufva and Allander 1995) and their numbers will increase during any immunological challenge (Ots and Hõrak 1998, Glaser and Kiecolt-Glaser 2005). Heterophils, as a part of the innate immune system, are non-specific phagocytozing cells entering the tissues during an inflammation, particularly due to microbial challenge (Campbell 1995). They increase in number during stress, trauma, and chronic bacterial infections. The H/L ratio is often used as a stress indicator in birds and is known to increase in situations of infectious diseases and/or starvation (Bonier et al. 2007, Davis et al. 2008).

As a measure of body reserves, we used size-adjusted body mass, a widely used and easy to measure index (Garnett 1981, Lindström and Piersma 1993, Alonso-Alvarez and Tella 2001). It provides the cleanest way to separate the effects of condition from the effects of body size (Jakob et al. 1996, Wojczulanis-Jakubas et al. 2015).

Laboratory work

To investigate BKC, we incubated 30 µl of fresh plasma with 10 µ of *E. coli* (American Type Culture Collection, strain 8739) at 41°C for 45 min following Matson et al. (2006). *Escherichia coli* was delivered in the form of freeze-dried pellets, with one pellet of *E. coli* containing a 5.5×10^7 colony forming unit. We made a stock solution with 50 ml of sterile 1M phosphate buffer saline (PBS) warmed to 35–37°C and one *E. coli* pellet, which we transferred to the PBS with sterile forceps. We prepared a working solution of *E. coli* by using 9.5 ml of sterile PBS to 0.5 ml of stock

solution, in order to produce a final starting concentration of a ~ 250 colony forming unit. We plated all samples on McConkey agar plates and stored them overnight in an incubator at 37°C to allow colony formation by surviving bacteria. As a control we plated and incubated 5 samples of bacteria without the addition of plasma.

To investigate leucocyte profiles, we stained air dried blood smears using the May–Grünewald–Giemsa method with a Wescor 'Aerospray Haematology' stainer. Then, we determined relative numbers by percentage of particular types of leucocytes by examining one-layer-cells on non-overlapping microscope fields of each smear at 1000 × magnification under oil immersion. We counted all types of leucocytes (heterophils, lymphocytes, basophiles, eosinophils and monocytes) until reaching a total of 100 cells (Davis et al. 2008). We used this data to calculate an H/L ratio. To estimate the overall allocation to leucocyte production, we counted the number of heterophils, lymphocytes and total leucocytes per 10 000 red blood cells (RBC) (Lobato et al. 2005). All blood smears were examined by a single observer who was unaware of the birds' breeding phase and sex (IK).

For molecular sexing of birds, we extracted DNA from the frozen red blood cells using a Blood Mini kit from A&A Biotechnology, Gdynia, Poland. We performed the CHD gene-based analysis with the primer pair F2550 and R2718, according to Griffiths et al. (1998), using a 50°C annealing temperature for the PCR reaction. The sex differences in the PCR products were clearly visible in UV-light when separating the fragments on 2% agarose gel stained with Midori Green Advanced DNA Stain (Nippon Genetics Europe, Düren, Germany).

Data analysis

We counted visible *E. coli* colonies on digital images of both the experimental (with plasma) and control (without plasma) plates using ImageJ 1.48v (National Inst. of Health, Bethesda, USA). The number of colonies in both types of plates were used to calculate BKC using the formula (Millet et al. 2007): BKC = 1 – (number of bacterial colonies on experimental plate/average number of bacterial colonies on 5 control plates).

Before statistical analyses, we logit-transformed the leucocyte variables and BKC (Warton and Hui 2011). Due to very low numbers or absence of monocytes, basophiles and eosinophils in the blood smears, we did not analyze them.

To adjust body mass to body size, we used the scaled mass index (SMI), as recommended by Peig and Green (2009):

$$SMI = Mi\left[\frac{Lo}{Li}\right]^{bSMA}$$

where Mi is the body mass of individual i; Li is the linear body measurement of individual i (head-bill length) and bSMA is the scaling exponent estimated from the regression of M and L. Lo is the arithmetic mean value of the linear measurement. We used mean value of head-bill length as the linear body size measurement as it showed the highest correlation with the body mass in adults (both sexes combined; Pearson correlation coefficient, $r_{120} = 0.38$, $p = 0.003$) among considered variables (bill length, $r_{120} = 0.18$, $p = 0.02$; wing length, $r_{120} = 0.14$, $p = 0.06$).

To investigate influence of phase of breeding season, sex, and their interaction on dependent variables (BKC, H/L ratio, numbers of heterophils, lymphocytes and all leucocytes per 10 000 RBC), we performed a factorial analysis of covariance (ANCOVA) with SMI as the covariant. We performed this separately for particular dependent variables. As the main effects should not be interpreted when interactions are significant (McDonald 2008), whenever that was the case, we interpreted only the interactions. We also performed factorial a ANOVA to investigate the influence of phase of breeding and sex and their interaction on the scaled mass index.

To examine relationships between variables, we performed a general linear model (GLM) using three different approaches to avoid multicollinearity of blood parameters. In the first approach, we included in the model: BKC as dependent variable and used the following set of non-collinear variables (Pearson correlation, $r < 0.31$): phase of breeding season as factorial predictor and H/L ratio, SMI as continuous predictors. In the second approach, we considered BKC as dependent variable and the following set of non-collinear variables ($r < 0.22$): phase of breeding season as factorial predictor and number of heterophils, lymphocytes and leucocytes per 10 000 RBC as continuous predictors. In order to determine the relationship between BKC and the values of H/L ratio, SMI, and sex at each studied stage phase of the breeding season, we additionally performed four separate multiple regression models. In the third approach, we considered SMI as the dependent variable and the following set of non-collinear variables: BKC, H/L ratio as continuous predictors and phase of breeding season as factorial predictor. In all regression analyses we coded sex as a dummy variable.

To select the best model for each set of regression analyses, we used Akaike's information criterion for small sample size (AICc) (Burnham and Anderson 2002, Mazerolle 2006). To compare the relative performance of the models, we calculated the difference (ΔAICc) between the AIC value of the best model and AIC value for each of the other models and Akaike's weights, i.e. probability that a model is the best model for observed data given the candidate set of models (Burnham and Anderson 2002). We considered only the models with $\Delta AICc < 2$ (Burnham and Anderson 2002). We checked significance of the best models using Wald statistics. To present effect size in the best models, we presented Eta-squared values.

We performed all statistical analyses in STATISTICA 10 (StatSoft, Tulsa, OK, USA), and R software (R Core Team) using MuMIn (Bartoń 2013) and aod (Lesnoff and Lancelot 2012) package. We considered results significant at level of $p < 0.05$ and for multiple comparisons we used Bonferroni correction with significance level $p < 0.01$.

Results

Influence of the phase of breeding season and sex on BKC, leukocyte profiles and SMI

Bacteria killing capacity (BKC) was affected significantly by the phases of breeding season with higher values at early phases

compared to the later phases (ANCOVA; post-hoc HSD test for unequal N with Bonferroni correction, $p < 0.01$, Table 1, Fig. 1). BKC was also affected significantly by a covariant, scaled mass index (SMI) with larger individuals having higher BKC. Neither sex nor interaction of sex with breeding phase affected BKC significantly (Table 1).

The H/L ratio was affected significantly by the breeding phase, sex, SMI and interaction of sex × phase of breeding (Table 1). Regarding interaction, the H/L ratio in both males and females was significantly higher during the early phases of breeding when compared to later ones (post-hoc HSD test for unequal N with Bonferroni correction, $p < 0.01$; Table 1, Fig. 2). In females, the H/L ratio was significantly higher at the beginning of incubation when compared to the chick rearing phase (post-hoc HSD test for unequal N with Bonferroni correction, $p < 0.01$; Fig. 2).

Numbers of heterophils, lymphocytes, leucocytes per 10 000 RBC and SMI (covariant) were also affected significantly by the breeding phase and the relative number of heterophils was affected by sex and phase × sex interaction (Table 2). Neither sex nor interaction with breeding phase affected significantly the number of lymphocytes and leucocytes per 10 000 RBC. Considering breeding phase effect, the number of all considered cells per 10 000 RBC was significantly higher during the early breeding phases when compared to later phases (post-hoc HSD test for unequal N with Bonferroni correction, $p < 0.01$; Fig. 2). Regarding breeding phase × sex interaction, during the prelaying phase males had a significantly higher number of heterophils per 10 000 RBC compared to females (post-hoc HSD test for unequal N with Bonferroni correction, $p < 0.01$).

The scaled mass index (SMI) was affected significantly by the breeding phase (ANOVA, $F_{1,114} = 13.34$, $p < 0.001$, Fig. 3) and sex ($F_{1,114} = 6.79$, $p = 0.011$). Interaction of breeding phase × sex was not significant ($F_{1,114} = 0.76$, $p = 0.519$). Individuals during chick rearing phase had lower SMI compared to the earlier phases. Overall, males had higher SMI than females (post-hoc HSD test for unequal N with Bonferroni correction, $p < 0.01$).

Relationships among the studied variables

The best model describing the relationship between BKC and the first set of predictors (breeding phase, H/L ratio, SMI and sex) included: breeding phase, SMI, H/L ratio, and sex (Table 3). BKC decreased with the progress of season ($p = 0.022$, Partial Eta-Squared = 0.65) and increased with increasing H/L ratio ($p = 0.009$, Partial Eta-Squared = 0.47) and with increasing SMI ($p = 0.004$, Partial Eta-Squared = 0.58). The sex effect was not significant ($p = 0.65$, Eta-squared = 0.18).

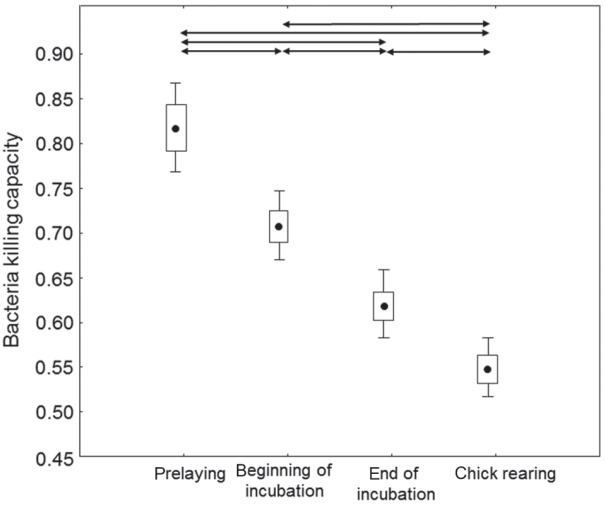

Figure 1. Bacteria killing capacity (points are means boxes are SD; whiskers are 95% CI, values corrected for significant covariant, scaled mass index) in adult little auks (both sex combined due to lack of significant sex differences) captured at the consecutive phases of breeding season. Arrows indicate significant differences (ANCOVA, post-hoc HSD test for unequal N with Bonferroni correction, $p < 0.01$).

The best model describing the relationship between BKC and the second set of predictors (breeding phase, sex, number of heterophils, lymphocytes and leucocytes per 10 000 RBC) included: number of heterophils, lymphocytes, leucocytes per 10 000 RBC and sex (Table 3). BKC increased with increasing number of heterophils per 10 000 RBC ($p = 0.0003$, Partial Eta-Squared = 0.55) and number of leucocytes per 10 000 RBC ($p < 0.001$, Partial Eta-Squared = 0.51). The sex effect was not significant ($p = 0.71$, Partial Eta-Squared = 0.18).

In separate multiple regression models describing factors affecting BKC during particular phases of the breeding season (prelaying, beginning of incubation, end of the incubation and during the chick rearing phase), H/L ratio and SMI were significant predictors in the best models (Table 4). During the prelaying phase BKC increased with increasing H/L ratio ($\beta = 0.34$, $p = 0.002$, Partial Eta-Squared = 0.58) and SMI ($\beta = 0.48$, $p = 0.0018$, Partial Eta-Squared = 0.62) (Fig. 4). At beginning of incubation, BKC increased with increasing H/L ratio ($\beta = 0.32$, $p = 0.014$, Partial Eta-Squared = 0.66). The SMI effect

Table 1. The effects of breeding phase, sex and their interaction on bacteria killing capacity and the H/L ratio in adult little auks, with scaled mass index as covariant (ANCOVA). Significant effects ($p < 0.05$) are bolded.

	Bacteria killing capacity				H/L ratio			
Variable	DF	F	p	Eta Squared	DF	F	p	Eta Squared
Intercept	1	765.98	**<0.001**	0.78	1	637.87	**<0.001**	0.82
Scaled mass index	1	18.98	**0.001**	0.35	1	26.98	**<0.001**	0.41
Phase	1	543.92	**<0.001**	0.63	1	211.23	**<0.001**	0.73
Sex	1	0.11	0.623	0.06	1	6.18	**0.006**	0.32
Sex × phase	1	2.45	0.154	0.02	1	7.34	**0.007**	0.28
Error	114				114			

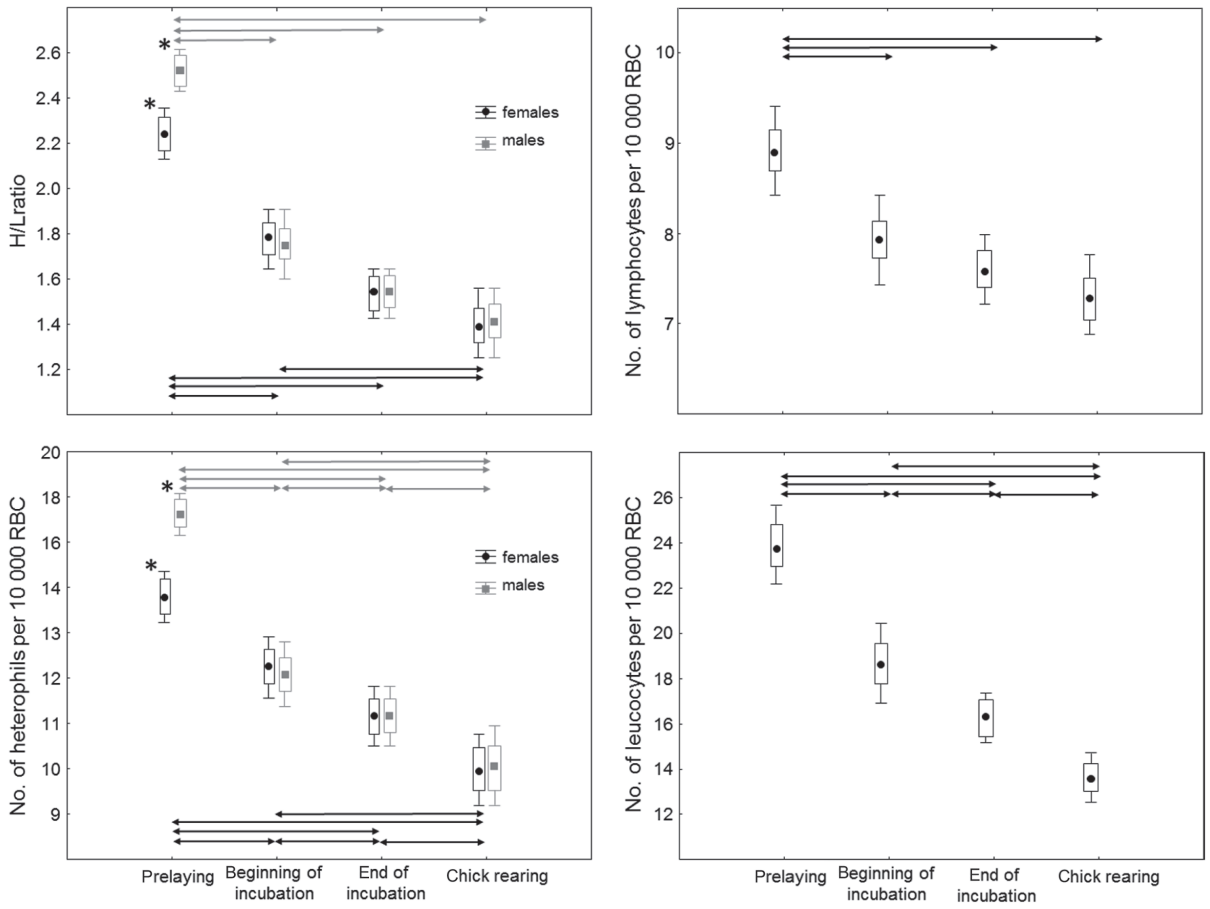

Figure 2. The H/L ratio, numbers of leucocytes, heterophils and lymphocytes per 10 000 RBC (points are means, boxes are SD, whiskers are 5–95% percentiles; values corrected for significant covariant, scaled mass index) in adult little auk males and females captured at the consecutive phases of breeding season. Arrows indicate significant differences among males, females or combined sexes and phases of breeding, stars indicate intersexual differences in particular phase (ANCOVA, post-hoc HSD test for unequal N with Bonferroni correction on logit transformed data, $p < 0.01$).

was not significant ($\beta = 0.16$, $p = 0.11$, Partial Eta-Squared = 0.21). At the end of the incubation phase, BKC increased with increasing H/L ratio ($\beta = 0.14$, $p = 0.028$, Partial Eta-Squared = 0.48). The SMI effect was not significant ($\beta = 0.13$, $p = 0.35$, Partial Eta-Squared = 0.18). During the chick rearing phase BKC increased with increasing H/L ratio ($\beta = 0.37$, $p = 0.022$, Partial Eta-Squared = 0.61) and SMI ($\beta = 0.31$, $p = 0.022$, Partial Eta-Squared = 0.43).

The best model describing the relationship between SMI and the set of predictors (BKC, H/L ratio, breeding phase, sex) included: BKC, breeding phase, sex. The SMI increased with increasing BKC ($p = 0.003$, Partial Eta-Squared = 0.57) and decreased with progress of the breeding season ($p < 0.001$, Partial Eta-Squared = 0.68). SMI was also influenced by sex ($p = 0.037$, Partial Eta-Squared = 0.47) with males being heavier (170.1 ± 6.2, $n = 62$) than females (167.1 ± 8.83, $n = 58$).

Discussion

We investigated changes in immunocompetence and body reserves with respect to the trade-off between reproduction and self-maintenance in the little auk. This is the first study on this species and one of the few conducted on seabirds examining this issue over the entirety of the breeding season, using multiple variables describing bird immunocompetence. We found that both immune function and size-adjusted body mass significantly declined with the progress of the breeding season regardless of sex.

The observed pattern of BKC changes in the little auk is consistent with the reproduction-immune system trade-off hypothesis (Hasselquist and Sherman 2001, Martin et al. 2008). The low values of BKC recorded during the chick rearing phase, when the cost related to reproduction is at maximum, may have resulted from suppression or down-regulation of the immunity (Hasselquist and Sherman 2001). Similar results have been reported for another seabird, the little penguin *Eudyptula minor*, where lower values of BKC were found during chick rearing compared to incubation phase (Evans et al. 2015).

In our study, BKC was positively correlated with SMI; values of both variables declined with the progress of the breeding season (Fig. 1 and 2), reflecting carry-over effects from previous phases of breeding (egg incubation, chick brooding and feeding). Previous studies have also reported lower SMI in adult little auks during chick rearing

Table 2. The effects of phase of breeding phase, sex, their interaction, with scaled mass index as covariant on numbers of heterophils and lymphocytes and total number of all leucocytes observed per 10 000 RBC in adult little auk (ANCOVA). Significant effects ($p < 0.05$) are bolded.

	Leucocytes				Heterophils			
Variable	DF	F	p	Eta-squared	DF	F	p	Eta-squared
Intercept	1	1543.11	**<0.001**	0.82	1	388.12	**<0.001**	0.77
Scaled mass index	1	307.54	**<0.001**	0.62	1	132.53	**<0.001**	0.46
Phase	1	187.64	**<0.001**	0.44	1	111.43	**<0.001**	0.41
Sex	1	4.11	0.119	0.23	1	6.43	**0.042**	0.26
Sex × phase	1	1.89	0.064	0.12	1	1.76	**0.005**	0.21

	Lymphocytes			
Variable	DF	F	p	Eta-squared
Intercept	1	98.34	**<0.001**	0.89
Scaled mass index	1	20.18	**<0.001**	0.47
Phase	1	48.34	**<0.001**	0.68
Sex	1	1.12	0.376	0.12
Sex × phase	1	2.54	0.097	0.18
Error	114			

compared to earlier phases of the breeding cycle (Taylor 1994, Wojczulanis-Jakubas et al. 2015, but see Wojczulanis-Jakubas et al. 2012). Mass loss during breeding, also observed in other alcids [e.g. in Atlantic puffins *Fratercula arctica* (Harris 1980), Brünnich's guillemots *Uria lomvia* (Gaston and Nettleship 1981)], is often interpreted as an indicator of the stress of reproduction (Ricklefs 1974). However, in our study, the H/L ratio declined throughout breeding season suggesting a decrease in stress level. An alternative hypothesis predicts that mass reduction in birds is adaptive. Lipid storage levels in birds may be minimized to maintain a low wing loading and to increase flight efficiency (Blem 1976, Norberg 1981). This may decrease energy expenditures during foraging to positively affect breeding success and survival of parent birds. Seasonal decrease in the body mass of little auks has been explained in before in terms of latter these hypothesis (Gabrielsen et al. 1991, Konarzewski et al. 1993). Although these hypotheses are not mutually exclusive because stress-induced loss of mass should lead to savings on energy expended on flight due to reduced wing loading,

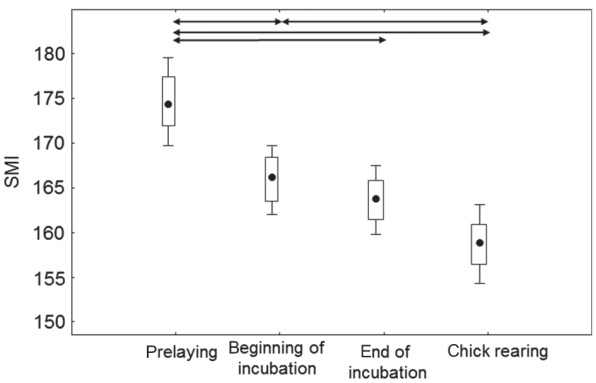

Figure 3. Scaled mass index (SMI) of adult little auks (both sex combined due to lack of sex differences) captured at the consecutive phases of breeding season (points are means; boxes are SD; whiskers are 5–95% percentiles). Arrows indicate significant differences (ANOVA, post-hoc HSD test for unequal N with Bonferroni correction, $p < 0.01$).

they differ in their predictions with respect to the phase of breeding with mass loss. If mass loss is stress-induced, we would expect a decrease in body mass throughout the breeding phase with the most rapid decline during the phase with the highest energetic demands (usually chick rearing) and this was recorded in our study. In contrast, if mass loss is adaptive, we would expect an abrupt, or stepwise decline in mass at or just before the moment when mass loss becomes most adaptive, i.e., when flight demand increases (Harding et al. 2004, Wojczulanis-Jakubas et al. 2012).

A positive relationship between BKC and SMI indicates that heavier individuals have a more effective innate branch of the immune system which is also reflected through higher numbers of leucocytes and heterophils per 10 000 RBC. This relationship of a more efficient immune system in heavier individuals has been previously reported in birds (Glick et al. 1983, Klasing 1988, Saino et al. 1997). In this context, our results suggest that lighter individuals are more challenged by reproduction, both at the beginning and the end of the breeding phase. Lighter individuals may also be less experienced in foraging and have to compromise immunocompetence as a cost of keeping their body mass at a sufficient level (Jakubas et al. 2012).

The result of individuals with higher H/L ratios having a higher BKC was unexpected as a high H/L level is usually attributed to a higher stress level that, in turn, is supposed to negatively affect immune system function (Davis et al. 2008). Results similar to those presented here have been reported for the red-winged blackbird *Agelaius phoeniceus*, in which BKC increased with stress levels, expressed as baseline corticosterone levels (Merrill et al. 2014). Parallel increase of BKC and H/L ratio may suggest mobilization of both branches of the immune system. In our study, this relationship was observed at all phases of the breeding phase despite the general decline in the value of H/L ratio through the course of the season. Moreover, during the final phase of breeding when they are exhausted, the heavier birds had a higher BKC. It is not likely that heavier birds with higher BKC values were more stressed (with higher H/L ratio) than lighter individuals. Thus, these results confirm our suggestion that increased values of BKC and H/L ratio indicate mobilization of both branches of the immune system. Our study indicates a trade-off between reproduction and self-maintenance (immune function and body mass) in little auks during the breeding season. BKC, numbers of leucocytes per 10 000 RBC and adjusted body mass in breeders declined with the progress of the breeding season, probably reflecting the carry-over effects from previous phases of breeding.

Table 3. The rank of the best linear models for factors affecting BKC and SMI based on Akaike information criterion corrected for small sample size – AICc; BreedPhase – breeding phase, Hete, Lymph, Leuco – number of heterophils, lymphocytes, leucocytes per 10 000 RBC.

Model: Studied variable ~ Predictors	DF	AICc	ΔAICc	Akaike's weights (w)	Wald test p
BKC ~ SMI, BreedPhase, Sex, H/L					
SMI + BreedPhase + H/L + Sex	114	336.6	0.0	0.28	<0.001
SMI + BreedPhase + H/L	115	338.7	3.7	0.18	0.007
SMI + BreedPhase	116	342.4	5.8	0.11	0.004
BKC ~ Sex, BreedPhase, Hete + Lymph + Leuco					
Hete + Lymph + Leuco + Sex + BreedPhase	115	256.9	0.0	0.22	<0.001
Sex + BreedPhase + Hete	116	267.8	7.1	0.18	<0.001
SMI ~ Sex, H/L, BKC, BreedPhase					
Sex + BKC + BreedPhase	114	178.3	0.0	0.36	<0.001
Sex + H/L + BKC + BreedPhase	113	179.8	8.1	0.26	<0.001
Sex + H/L + BreedPhase	114	181.8	10.1	0.21	0.006
Sex + H/L	115	189.9	11.6	0.14	0.004

Table 4. The rank of the best OLS regression models for factors affecting BKC separately for four phases of breeding season based on Akaike information criterion corrected for small sample size – AICc.

Model: Studied variable ~ Predictors	DF	AICc	ΔAICc	Akaike's weights (w)	Wald test p
Prelaying: BKC ~ SMI, H/L, Sex					
SMI + H/L	24	116.7	0.0	0.31	<0.001
SMI + H/L + Sex	23	124.8	8.1	0.12	0.011
H/L + Sex	24	131.2	14.5	0.16	0.033
Beginning of incubation: BKC ~ SMI, H/L, Sex					
SMI + H/L	30	148.6	0.0	0.26	0.003
SMI + H/L + Sex	29	159.3	10.7	0.13	0.043
End of the incubation: BKC ~ SMI, H/L, Sex					
SMI + H/L	36	104.2	0.0	0.27	0.007
SMI + H/L + Sex	35	113.9	9.7	0.09	0.037
Chick rearing: BKC ~ SMI, H/L, Sex					
SMI + H/L	26	98.2	0.0	0.35	<0.001
SMI + H/L + Sex	25	109.9	11.7	0.19	<0.001

Lack of sex differences in BKC, SMI and blood parameters (H/L ratio, numbers of heterophils, lymphocytes and all leucocytes per 10 000 RBC) throughout the breeding season deny the hypothesis that little auk females desert the brood earlier than the male because of body reserve depletion. Previous studies on temporal variation of body condition variables also do not support this hypothesis (Jakubas et al. 2008, Wojczulanis-Jakubas and Jakubas 2012, Wojczulanis-Jakubas et al. 2012, 2015).

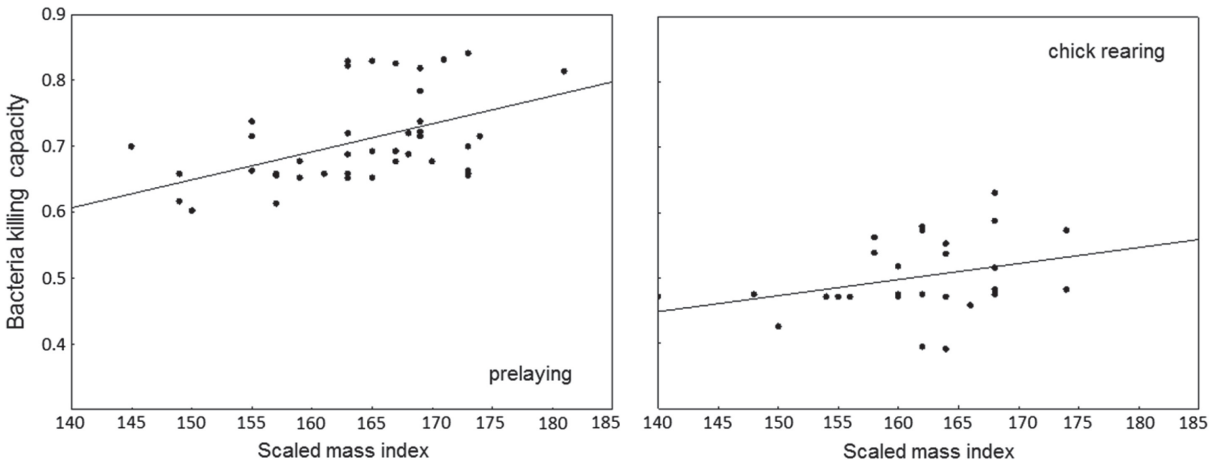

Figure 4. Relationship between the values of bacteria killing capacity and scaled mass index at the two phases of breeding which for the relationship was significant: prelaying (partial regression coefficient; $\beta = 0.42$, $p = 0.021$, $n = 25$) and chick rearing (partial regression coefficient; $\beta = 0.38$, $p = 0.035$, $n = 27$) in adult little auks. Least squares regression lines are shown for significant relationships.

Nevertheless, decreased immune function of a parental bird may affect the chick care strategy of the little auk, especially during transition to paternal-only care at the end of the chick rearing phase. Despite similar conditions at the end of breeding expressed by body mass, corticosterone and prolactin level (Wojczulanis-Jakubas et al. 2012, 2014, 2015) and similar allocation from self-maintenance to reproduction (this study), females may be more prone to elevated investments, and a consequence may be predisposed for brood desertion more than males due to the additive effect of all parental investments, including egg production (Wojczulanis-Jakubas et al. 2014). Moreover, long-lived species such as the little auk are predicted to restrict their current reproductive investment because even a small reduction in survival will have a large negative impact on lifetime reproductive output (Charlesworth 1980).

In conclusion, our study indicates that immunocompetence of the adult little auks decreases with the progress of the breeding season. This coincides with a decrease in body mass. Both processes are associated with re-allocation of energy from self-maintenance to reproduction. This suggests that reproduction takes place at the expense of other organismal systems, which may also have an impact on future survival. Lack of sex differences in BKC and SMI throughout the breeding season indicates similar parental investments of males and females. Our study shows that little auks may modulate their immune response and body mass as an adaptive life-history strategy for maximizing current reproduction. Further studies are needed to characterize the roles of the individual (e.g. age, experience, health status), environmental (e.g. local food conditions) and spatial (e.g. carry-over effects from wintering quarters) effects on the re-allocation size and how current re-allocation affect breeding success and adult survival.

Acknowledgements – We thank Wojciech Glac for staining of blood smears. All field work was done under the permission of the Norwegian Animal Research Committee and the Governor of Svalbard. The study was supported by a grant from National Science Centre (Preludium 5; UMO-2013/09/N/NZ8/03208) and by a grant from Univ. of Gdańsk (538-L120-B589-14).

References

Adamo, S. A., Jensen, M. and Younger, M. 2001. Changes in lifetime immunocompetence in male and female *Gryllus texensis* (formerly G-integer): trade-offs between immunity and reproduction. – Anim. Behav. 62: 417–425.

Alonso-Alvarez, C. and Tella, J. L. 2001. Effects of experimental food restriction and body-mass changes on the avian T-cell-mediated immune response. – Can. J. Zool. 79: 101–105.

Ankney, C. D. and Macinnes, C. D. 1978. Nutrient reserves and reproductive performance of female lesser snow geese. – Auk 95: 459–471.

Bartoń, K. 2013. MuMIn: multi-model inference, R package version 1.10.0. – <http://CRAN.R-project.org/package=MuMIn>.

Blem, C. R. 1976. Patterns of lipid storage and utilization in birds. – Am. Zool. 16: 671–684.

Bonier, F., Martin, P. R., Sheldon, K. S., Jensen, J. P., Foltz, S. L. and Wingfield, J. C. 2007. Sex-specific consequences of life in the city. – Behav. Ecol. 18: 121–129.

Burnham, K. P. and Anderson, D. R. 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd ed. – Springer.

Campbell, T. W. 1995. Avian hematology and cytology. – Iowa State Univ. Press.

Charlesworth, B. 1980. The evolution of age-structured populations. – Cambridge Univ. Press.

Davis, A. K. 2005. Effects of handling time and repeated sampling on avian white blood cell counts. – J. Field Ornithol. 76: 334–338.

Davis, A. K., Maney, D. L. and Maerz, J. C. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. – Funct. Ecol. 22: 760–772.

Deerenberg, C., Arpanius, V., Daan, S. and Bos, N. 1997. Reproductive effort decreases antibody responsiveness. – Proc. R. Soc. B 264: 1021–1029.

Dehnhard, N. and Hennicke, J. C. 2013. Leucocyte profiles and body condition in breeding brown boobies and red-tailed tropicbirds: effects of breeding stage and sex. – Aust. J. Zool. 61: 178–185.

Dehnhard, N., Poisbleau, M., Demongin, L. and Quillfeldt, P. 2011. Do leucocyte profiles reflect temporal and sexual variation in body condition over the breeding cycle in southern rockhopper penguins? – J. Ornithol. 152: 759–768.

Dein, J. 1986. Hematology. – In: Harrison, G. J. and Harrison, L. R. (eds), Clinical avian medicine and surgery. Saunders, pp. 174–191.

Drent, R. H. and Daan, S. 1980. The prudent parent: energetic adjustments in avian breeding. – Ardea 68: 225–252.

Dufva, R. and Allander, K. 1995. Intraspecific variation in plumage coloration reflects immune response in great tit (*Parus major*) males. – Funct. Ecol. 9: 785–789.

Evans, J. K., Dann, P. and Frankel, T. 2015. Variation in innate immune function during incubation, chick-rearing and moult in little penguins (*Eudyptula minor*). – Emu 115: 63–71.

Gabrielsen, G. W., Taylor, J. R., Konarzewski, M. and Mehlum, F. 1991. Field and laboratory metabolism and thermoregulation in dovekies (*Alle alle*). – Auk 108: 71–78.

Garnett, M. C. 1981. Body size, its heritability and influence on juvenile survival among great tits, *Parus major.* – Ibis 123: 31–41.

Gaston, A. J. and Nettleship, D. N. 1981. The thick-billed murres of Prince Leopold Island. – Canadian Wildlife Service no. 6, Ottawa.

Glaser, R. and Kiecolt-Glaser, J. K. 2005. Stress-induced immune dysfunction: implications for health. – Nat. Rev. Immunol. 5: 243–251.

Glick, B., Taylor, R. L., Martin, D. E., Watabe, M., Day, E. J. and Thompson, D. 1983. Calorie-protein deficiencies and the immune response of the chicken. II. Cell-mediated immunity. – Poult. Sci. 62: 1889–1893.

Griffiths, R., Double, M. C., Orr, K. and Dawson, R. J. G. 1998. A DNA test to sex most birds. – Mol. Ecol. 7: 1071–1075.

Harding, A. M. A., Pelt, T. I. V., Lifjeld, J. T. and Mehlum, F. 2004. Sex differences in little auk *Alle alle* parental care: transition from biparental to paternal-only care. – Ibis 146: 642–651.

Harris, M. P. 1980. Breeding performance of puffins *Fratercula arctica* in relation to nest density, laying date and year. – Ibis 122: 193–209.

Hasselquist, D. and Sherman, P. W. 2001. Social mating systems and extrapair fertilizations in passerine birds. – Behav. Ecol. 12: 457–466.

Jaeger, A., Goutte, A., Lecomte, V. J., Richard, P., Chastel, O., Barbraud, C., Weimerskirch, H. and Cherel, Y. 2014. Age, sex, and breeding status shape a complex foraging pattern in an extremely long-lived seabird. – Ecology 95: 2324–2333.

Jakob, E. M., Marshall, S. D. and Uetz, G. W. 1996. Estimating fitness: a comparison of body condition indices. – Oikos 77: 61–67.

Jakubas, D. and Wojczulanis-Jakubas, K. 2013. Rates and consequences of relaying in little auks *Alle alle* breeding in the High Arctic an experimental study with egg removal. – J. Avian Biol. 44: 62–68.

Jakubas, D., Wojczulanis-Jakubas, K. and Kreft, R. 2008. Sex differences in body condition and hematological parameters in little auk *Alle alle* during the incubation phase. – Ornis Fenn. 85: 90–97.

Jakubas, D., Iliszko, L., Wojczulanis-Jakubas, K. and Stempniewicz, L. 2012. Foraging by little auks in the distant marginal sea ice zone during the chick-rearing period. – Polar Biol. 35: 73–81.

Klasing, K. C. 1988. Influence of acute feed deprivation or excess food intake on immuno-competence of broiler chicks. – Poult. Sci. 67: 626–634.

Konarzewski, M., Taylor, J. R. and Gabrielsen, G. W. 1993. Chick energy requirements and adult energy expenditures of dovekies (*Alle alle*). – Auk 110: 343–353.

Lesnoff, M. and Lancelot, R. 2012. aod: analysis of overdispersed data. – R package ver. 1.3, <http://cran.r-project.org/package=aod>.

Lindström, Å. and Piersma, T. 1993. Mass changes in migrating birds: the evidence for fat and protein storage re-examined. – Ibis 135: 70–78.

Lobato, E., Moreno, J., Merino, S., Sanz, J. J. and Arriero, E. 2005. Haematological variables are good predictors of recruitment in nestling pied flycatchers (*Ficedula hypoleuca*). – Ecoscience 12: 27–34.

Martin, L. B., Weil, Z. M. and Nelson, R. J. 2008. Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. – Phil. Trans. R. Soc. B 363: 321–339.

Martin, T. E. 1987. Food as a limit on breeding birds: a life-history perspective. – Annu. Rev. Ecol. Syst. 18: 453–487.

Matson, K. D., Tieleman, B. I. and Klasing, K. C. 2006. Capture stress and the bactericidal competence of blood and plasma in five species of tropical birds. – Physiol. Biochem. Zool. 79: 556–564.

Mazerolle, M. J. 2006. Improving data analysis in herpetology: using Akaike's information criterion (AIC) to assess the strength of biological hypotheses. – Amphibia-Reptilia 27: 169–180.

McDonald, P. 2008. Primordial non-Gaussianity: large-scale structure signature in the perturbative bias model. – Phys. Rev. 78: 123–519.

Merchant, M. E., Pallansch, M., Paulman, R. L., Wells, J. B., Nalca, A. and Ptak, R. 2005. Antiviral activity of serum from the American alligator (*Alligator mississippiensis*). – Antiviral Res. 66: 35–38.

Merrill, L., Levinson, S. D., O'Loghlen, A. L., Wingfield, J. C. and Rothstein, S. I. 2014. Bacteria-killing ability is negatively linked to epaulet size, but positively linked to baseline corticosterone, in male red-winged blackbirds (*Agelaius phoeniceus*). – Auk 131: 3–11.

Millet, S., Bennett, J., Lee, K. A., Hau, M. and Klasing, K. C. 2007. Quantifying and comparing constitutive immunity across avian species. – Develop. Comp. Immunol. 31: 188–201.

Norberg, R. A. 1981. Temporary weight decrease in breeding birds may result in more fledged young. – Am. Nat. 118: 838–850.

Ochsenbein, A. F., Fehr, T., Lutz, C., Suter, M., Brombacher, F., Hengartner, H. and Zinkernagel, R. M. 1999. Control of early viral and bacterial distribution and disease by natural antibodies. – Science 286: 2156–2159.

Ots, I. and Hõrak, P. 1998. Health impact of blood parasites in breeding great tits. – Oecologia 116: 441–448.

Peig, J. and Green, A. J. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. – Oikos 118: 1883–1891.

Powell, L. L., Dobbs, R. C. and Marra, P. P. 2015. Habitat and body condition influence American redstart foraging behaviour during the non-breeding season. – J. Field Ornithol. 86: 229–237.

Ricklefs, R. E. 1974. Energetics of reproduction in birds. Avian energetics. – Nuttal Ornithol. Club 15: 152–297.

Saino, N., Bolzern, A. M. and Møller, A. P. 1997 Immuno-competence, ornamentation and viability of male barn swallows (*Hirundo rustica*). – Proc. Natl Acad. Sci. USA 94: 579–585.

Salvante, K. G. 2006. Techniques for studying integrated immune function in birds. – Auk 123: 575–586.

Sandberg, R. and Moore, F. R. 1996. Fat stores and arrival on the breeding grounds: reproductive consequences for passerine migrants. – Oikos 77: 577–581.

Sol, D., Timmermans, S. and Lefebvre, L. 2002. Behavioural flexibility and invasion success in birds. – Anim. Behav. 63: 495–502.

Stempniewicz, L. 1995. Predator–prey interactions between glaucous gull *Larus hyperboreus* and little auk *Alle alle* in Spitsbergen. – Acta Ornithol. 29: 155–170.

Stempniewicz, L. 2001. *Alle alle* little auk. – J. Birds Western Palearctic 3: 175–201.

Taylor, J. R. E. 1994. Changes in body mass and body reserves of breeding little auks (*Alle alle*). – Polish Polar Res. 15: 147–168.

Walsberg, G. E. 1983. Avian ecological energetics – avian biology. – Academic Press.

Warton, D. I. and Hui, F. K. 2011. The arcsine is asinine: the analysis of proportions in ecology. – Ecology 92: 3–10.

Wojczulanis-Jakubas, K. and Jakubas, D. 2012. When and why does my mother leave me? The question of brood desertion in the dovekie (*Alle alle*). – Auk 129: 632–637.

Wojczulanis-Jakubas, K., Jakubas, D., Kidawa, D. and Kośmicka, A. 2012. Is the transition from biparental to male-only care in a mono-gamous seabird related to changes in body mass and stress level? – J. Ornithol. 153: 793–800.

Wojczulanis-Jakubas, K., Jakubas, D., Kulaszewicz, I., Kidawa, D. and Taylor, J. R. E. 2014. Influence of primary reproductive investments on blood biochemistry, leukocyte profile, and body mass in a small Arctic seabird. – Auk 131: 743–755.

Wojczulanis-Jakubas, K., Jakubas, D., Chastel, O. and Kulaszewicz, I. 2015. A big storm in a small body: seasonal changes in body mass, hormone concentrations and leukocyte profile in the little auk (*Alle alle*). – Polar Biol. 38: 1203–1212.