

Reproductive investment is connected to innate immunity in a long-lived animal

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Abstract Life-history theory predicts that organisms optimize their resource allocation strategy to maximize lifetime reproductive success. Individuals can flexibly reallocate resources depending on their life-history stage, and environmental and physiological factors, which lead to variable life-history strategies even within species. Physiological trade-offs between immunity and reproduction are particularly relevant for long-lived species that need to balance current reproduction against future survival and reproduction, but their underlying mechanisms are poorly

understood. A major unresolved issue is whether the first-line innate immune function is suppressed by reproductive investment. In this paper, we tested if reproductive investment is associated with the suppression of innate immunity, and how this potential trade-off is resolved depending on physiological state and residual reproductive value. We used long-lived capital-breeding female eiders (*Somateria mollissima*) as a model. We showed that the innate immune response, measured by plasma bacteria-killing capacity (BKC), was negatively associated with increasing reproductive investment, i.e., with increasing clutch size and advancing incubation stage. Females in a better physiological state, as indexed by low heterophil-to-lymphocyte (H/L) ratios, showed higher BKC during early incubation, but this capacity decreased as incubation progressed, whereas females in poorer state showed low BKC capacity throughout incubation. Although plasma BKC generally declined with increasing H/L ratios, this decrease was most pronounced in young females. Our results demonstrate that reproductive investment can suppress constitutive first-line immune defence in a long-lived bird, but the degree of immunosuppression depends on physiological state and age.

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In this study we have investigated whether innate immune function is suppressed by the reproductive investment in a long-lived bird. We demonstrate for the first time that the innate immune response, measured as bacteria killing capacity of blood plasma, is negatively associated with increased reproductive investment in a wild bird. However, the degree of suppression of this first line of immune defence depends on both the health status and age of the bird.

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Introduction

The fitness of long-lived animals is highly dependent on survival, wherefore self-maintenance and survival are expected to be prioritized over current reproduction (Drent and Daan 1980; Stearns 1992). However, as animals age

their residual reproductive value decreases, which may necessitate a shift towards prioritizing reproduction (Pianka and Parker 1975). Such increased investment into current reproduction increases the likelihood of offspring surviving to reproductive age. However, this may entail a cost for the reproducing individual. To allocate resources to reproduction, some costly physiological functions may need to be suppressed even when it threatens survival (Deerenberg et al. 1997; Hanssen et al. 2003). Although differences in individual state can help to explain how these trade-offs between current reproduction and survival are resolved (e.g., Weladji et al. 2008; Hamel et al. 2009), the underlying mechanisms of this variation are yet to be discovered.

The costs of maintaining a functional immune defence, and those of mounting an immune response, may interfere with the other important physiological pathways, such as those associated with reproduction (Sheldon and Verhulst 1996; Norris and Evans 2000; Hasselquist and Nilsson 2012). To maintain high reproductive output, one would, therefore, expect the immune system to be suppressed and resources reallocated to other functions particularly during the breeding season (e.g., Mann et al. 2000; Kortet et al. 2003). Nevertheless, resource allocation to the immune system is expected to vary between individuals depending on the relative emphasis that they place on survival versus current reproduction (e.g., Ardia 2005a). Individuals with higher residual reproductive value are expected to invest more into immune function and hence, survival, whereas older individuals, with limited remaining breeding opportunities ahead of them, are expected to prioritize current reproduction at the cost of self-maintenance (Cichoń 2001; Velando et al. 2006). Even though a trade-off between immunity and reproductive investment has been confirmed by a growing number of empirical studies (e.g., Ilmonen et al. 2000; Råberg et al. 2000; Cichoń et al. 2001), the lack of it has also been reported (e.g., Williams et al. 1999). This inconsistency may stem from differences in the component of the immune system under test, the type of manipulation as well as from species-specific differences in resource allocation (Yang et al. 2013).

The innate branch of the immune system acts as the first-line immediate defence against foreign organisms, playing a central role in triggering acquired immune responses (Janeway et al. 2001), thus serving as a key defence mechanism contributing to survival and, ultimately, to fitness (Lochmiller and Deerenberg 2000). Given the presumed pivotal role of innate immunity in the development of adaptive immunity, it is surprising that evidence for the suppression of innate immunity in response to increased reproductive investment still remains rather scarce and controversial (e.g., Bourgeon et al. 2007; French and Moore 2008). Any suppression of innate immunity during breeding may depend on both the individual's condition and the external

environment (French and Moore 2008; Downs et al. 2015). Investigating state-dependent investment strategies between innate immunity and reproduction may reveal how different life-history strategies are related to this key trade-off (e.g., Downs et al. 2015).

There is a growing body of literature examining whether immunity-reproduction trade-offs can be detected in wild animals living in their natural environment, particularly in the field of adaptive immunity (e.g., Ruiz et al. 2011). However, such studies are still relatively rare in comparison to those conducted in the laboratory model systems using domesticated animals, or investigating wild animals subjected to sometimes inadequate immune challenge experiments (Maizels and Nussey 2013).

Here, we explore whether individuals investing more resources into reproduction have lower innate immune function, and whether this association is modulated by individual state and residual reproductive value. We predicted (1) a decrease in immune function with increasing reproductive investment. Because reproductive effort and immune function are likely to compete for the same limited resources, only high-quality individuals may afford to divert resources away from the immune system to maximize their reproductive success without detrimental effects on their health (e.g., Ardia et al. 2003; Ardia 2005b; Downs et al. 2015). Thus, high-quality individuals may be well above the threshold level of immune competence allowing for adequate pathogen defence at the onset of breeding, and hence can afford to down-regulate immune defences during the course of the breeding event to accommodate the increased reproductive demands (Hamilton and Zuk 1982; Andersson 1994). We, therefore, predict that (2) individuals in better physiological state are more likely to down-regulate their immune defences during a reproductive event. Alternatively, however, high-quality individuals may be able to acquire more resources and may thus allocate more energy, simultaneously, in both reproduction and immune function than low-quality individuals (cf. Wilson and Nussey 2010). We also predicted that (3) residual reproductive value, which typically declines with age (Stearns 1992; Cotter et al. 2011), affects the allocation of resources between survival (i.e., immune function) and reproduction, so that individuals down-regulate immune functions with advancing age to facilitate increased reproductive effort.

To test these predictions, we conducted a cross-sectional study on a breeding population of eider females (*Somateria mollissima*). This study species exhibits several attractive features for investigating potential trade-offs between reproductive investment and immune function. First, eiders are long-lived with an estimated life expectancy exceeding 20 years (Baillie and Milne 1982; Coulson 1984), emphasizing the importance of the trade-off between reproduction and survival. Second, eiders invest significant amounts

of stored energy in producing typically 3–6 eggs (Parker and Holm 1990; Hobson et al. 2015). Third, females also rely solely on stored energy reserves during their 26-day incubation period when they consequently lose a significant proportion (up to 40 %) of their body mass (Parker and Holm 1990; Hanssen et al. 2002). This emphasizes the finitude of resources available for the incubating female and the importance of balancing investment in the current reproduction against future reproductive prospects. Finally, the immune function of eiders may be down-regulated during the incubation fast (Hanssen et al. 2005), despite the presence of a range of parasites and pathogens (Hollmén 2002; Borgsteede et al. 2005; Taylor et al. 2007) with potentially severe fitness consequences, even leading to the death of adults or offspring (e.g., Swennen and Smit 1991; Hollmén et al. 1999; Borgsteede 2005). We analysed how innate immunity of incubating female eiders is associated with reproductive investment, age, and physiological state. We assessed innate immunity by bacteria-killing capacity (BKC) of blood plasma, an increasingly popular technique which quantifies the integrated capacity of plasma proteins (e.g., complement, lysozyme) to kill bacteria (Ricklin et al. 2010; Nebel et al. 2013; Merrill et al. 2014). Complement protein activity, which is responsible for most of the bactericidal abilities of the plasma (Matson et al. 2006), is a key component of innate immunity and a crucial element for the development of adaptive immune responses (Janeway et al. 2001; Morgan et al. 2005). Clutch size and the number of days the female had incubated the clutch (incubation stage) measured reproductive investment, female minimum breeding experience was estimated from ringing data, and physiological state was indexed by the body condition and the heterophil-to-lymphocyte (H/L) ratio. An elevated H/L ratio may indicate recent exposure to physiological stress (Maxwell 1993; Lütke et al. 2013) or increased reproductive effort (reviewed by Davis et al. 2008), and is thereby a good proxy for physiological state.

Materials and methods

Study area and field methods

This study was conducted at Tvärminne Zoological Station (59°50'N, 23°15'E), western Gulf of Finland, during May 2013. In this study area, females nest on small open and larger forested islands. Nesting females ($N = 137$) were captured with hand nets during the latter half of the incubation period (mean \pm SD = 16.2 \pm 6.0 days of incubation). The duration of each trapping session was restricted to less than 90 min to reduce disturbance and the risk of nest desertion (Bolduc and Guillemette 2003). Upon capture, females were weighed, measured for structural size (length

of the radius-ulna), and first-time breeders ($N = 54$) were ringed with standard metal rings. Females in the study population are not ringed as ducklings. If the captured female was previously ringed ($N = 83$), the number of years, since it was ringed was, therefore, used as a relative age indicator estimating (minimum) years of breeding experience (Öst et al. 2008a; Jaatinen and Öst 2011). Although this measure is correlated with age, it inevitably incorporates some measurement error stemming from variation in the age at first breeding. Nevertheless, variation in the age at first breeding can be considered small (2–5 years; Hario and Rintala 2009) in comparison to the range of (minimum) years of breeding experience in this study (0–16 years; $N = 137$). Thereby, our minimum age estimate is a reasonably accurate indicator of breeding experience, and this is further underlined by the facts that females are highly philopatric to their breeding islands (Öst et al. 2011) and that more than half of the successfully breeding females are trapped annually with a fairly constant trapping effort since 1996 (Jaatinen and Öst 2011). Females in this study had an average breeding experience of 2.8 years (± 3.83 SD; $N = 137$).

Clutch size, which was recorded upon female capture, is a good measure of reproductive investment, since females utilize stored energy reserves for egg production (Parker and Holm 1990; Hobson et al. 2015), and the frequency of conspecific brood parasitism is low in this population (Waldeck et al. 2004). Incubation stage was determined by egg floatation (Kilpi and Lindström 1997). Incubation stage reflects the amount of time and energy; a female has invested in the current reproductive attempt; the reproductive value of the clutch increases with time spent incubating. Thus, incubation stage was used as another estimate of reproductive investment. Upon capture, blood samples (<1 ml) were collected from the brachial vein. Blood smears for leukocyte counts were immediately prepared and fixed in 100 % methanol. Collected blood samples were stored on ice for a maximum of 4 h until transported to the laboratory, where plasma was separated by centrifugation at 1500 \times g for 10 min and stored at -80 °C until further analysis.

Measuring individual state: body condition

We used residual body mass relative to body size as an index of body condition, since fat reserves show a strong positive correlation with total body mass in eiders (Jamieson et al. 2006). We calculated a body condition index for all trapped females provided that they had incubated their eggs for >8 days (egg laying may otherwise still be in progress; Öst et al. 2008b). This was done by regressing the log-transformed projected body mass at hatching against the log-transformed radius-ulna length and using

the standardized residuals as a condition index. The projected body mass at hatching was obtained by subtracting the expected body mass loss (by calculating the expected number of days before hatching based on incubation stage) from the body mass measured at capture. We caught females at different incubation stages, which enabled us to quantify average body mass loss by regressing the logarithm of body mass against the logarithm of incubation stage and projected hatching date (Öst et al. 2008b).

Measuring individual state: leucocyte counts

Methanol-fixed blood smears were stained with Giemsa and examined under a 1000 \times magnification. The first 100 leucocytes counted were identified, and based on these, the H/L ratio was calculated. Due to the similar morphology of heterophils and eosinophils in the stained blood samples and the difficulty to distinguish between them (Campbell 1988), we pooled the number of these cells. The proportion of circulating eosinophils in avian blood is low, and constitutes only 0–6 % of all leucocytes (Davis et al. 2008) and pooling these cells, a method previously validated by Clinchy et al. (2004), should, therefore, not introduce any serious bias. The mean H/L ratio was 1.068 (± 0.98 SD; $N = 113$). To calculate the repeatability of H/L measurements, we randomly selected 15 blood slides and counted the first 100 leucocytes three times for each slide and used these data in the repeatability analysis (Wolak et al. 2012). Thus, the statistical significance of repeatability was estimated based on a likelihood ratio test (LRT) comparing a linear mixed effect model containing a grouping random factor (viz., female identity) to a model without this factor. The repeatability of the H/L ratio counts within samples was moderate ($r = 0.38$) and significant (LRT = 5.35, $df = 3$, $P = 0.02$, $N = 15$).

Innate immunity: plasma bacteria-killing capacity

The BKC assay was performed only for plasma samples collected during the same hours ($N = 51$). This was done, because the project initially also aimed at analysing the prevalence of coccidians showing diel periodicity (Villanúa et al. 2006); however, the low prevalence of these parasites precluded further analysis. The BKC assay was performed in a sterile laminar flow using a strain of *Escherichia coli* (ATCC no. 8739, Leibniz-Institut DSMZ GmbH) that is specifically lysed by the complement (method modified from Millet et al. 2007; Liebl and Martin 2009). The *E. coli* strain, supplied lyophilized as 10^7 – 10^8 organisms per pellet, was reconstituted by dissolving the pellet in 1-ml sterile phosphate buffered saline (PBS) to make a stock solution. The bacteria stock was diluted

1:100 in PBS, and plasma samples were diluted 1:10 in PBS. One part of *E. coli* solution was added to five parts of diluted plasma or positive control samples (containing only PBS) and incubated at +37 °C for 30 min to induce bacterial killing. Samples were then plated on Luria Agar plates, 50 μ l of each sample on duplicate plates. Positive control plates were made prior to (“before”) and at the end of (“after”) the sample plating, to assess and control for possible bacterial growth during the plating protocol. No changes in bacterial growth between the duplicate “before” and “after” control plates were detected (paired t test: $t = 0.86$, $P = 0.48$, $N = 3$). All plates were covered and incubated upside down for 16 h at +37 °C. The total number of colony-forming units (CFUs) was counted from each plate the following morning, and BKC was calculated as the mean number of CFUs in each sample divided by the mean number of CFUs in the positive controls. This fraction was subtracted from 1 to obtain the proportion of bacteria lysed by the complement. This protocol induced marked reduction in bacteria numbers with a mean BKC value of 0.41 (± 0.33 SD; $N = 51$).

Statistical analyses

We analysed variation in plasma BKC using a generalized linear model (GLM) with clutch size, incubation stage, body condition, the H/L ratio, and minimum breeding experience as covariates. Due to missing values for some of the explanatory variables and sample size limitations regarding BKC (see above), the final sample size of females was reduced to 35. Because of this limited sample size, we refrained from testing all possible two-way interactions and focused only on the essential interactions involving female state (the H/L ratio, body condition, and breeding experience) and the other variables in the model (Table 1). To test the robustness of our body condition index, we also constructed a GLM that included residual body mass at sampling, corrected for female structural size, as a covariate instead of body condition at hatching (see above). Using this alternative, covariate yielded the same final model, and the results were qualitatively identical to the analysis reported here (Table S1).

Six out of the 35 samples yielded negative BKC values. Since such values indicate an increase in bacteria numbers, we performed a validation analysis to ensure the data did not contain spurious observations. To this end, we re-ran the same GLM model, but replacing negative values with zeros. The results were qualitatively and quantitatively almost identical to the results obtained using negative BKC values. To conclude, the negative values represented true observations of individuals with considerably low BKC and the slightly negative values observed merely represented random measurement error.

Table 1 Model selection and GLM to test the effect of a set of independent variables on bacteria-killing capacity (BKC) of blood plasma of incubating eider females

Response variable	Independent variables	<i>b</i>	SE	<i>t</i>	<i>df</i>	<i>P</i>
BKC	Clutch size	-0.11	0.04	-2.56	28	0.02
	Incubation stage	-0.11	0.04	-3.22	28	<0.01
	Age	-0.08	0.03	-2.49	28	0.02
	H/L	-2.42	0.85	-2.87	28	<0.01
	Age × H.L	0.09	0.03	2.85	28	<0.01
	Incubation stage × H/L	0.10	0.04	2.82	28	<0.01
	Body condition	-0.08	0.06	-1.28	25	0.21
	Clutch size × H/L	0.16	0.19	0.81	24	0.42
	Body condition × H/L	-0.30	0.44	-0.69	23	0.50

Variables and two-way interactions represented in bold were significant ($\alpha = 0.05$) and included in the final model

b parameter estimate, *SE* standard error, *df* degrees of freedom

Variance inflation factors (VIFs) for the independent variables were less than 1.1 and thus collinearity between predictors is unlikely to affect our results (Neter et al. 1996). To graphically illustrate significant interaction terms, these were analysed post-hoc using the established method of simple slope analysis (Aiken and West 1991). In short, regression equations were restructured to reflect the regression of the criterion on one predictor, and simple slope regressions were plotted to display the interactions at the 25th, the 50th (median), and the 75th percentile.

All non-significant interactions and variables were sequentially removed from the final model ($\alpha = 0.05$; Table 1). The residuals of the model adhered to the assumption of normality. All statistical analyses were performed using the statistical software R (R Core Team 2013).

Results

Female BKC was explained by clutch size and by the interactions between H/L and incubation stage and H/L and female minimum age. The final model (Table 1) explained a relatively large portion of the variation in BKC (GLM: $F_{6,28} = 4.20$, $R^2 = 0.47$, $P = 0.004$). BKC showed a negative association with both clutch size ($b = -0.11$, $t = -2.56$, $df = 28$, $P = 0.02$; Table 1; Fig. 1) and advancing incubation stage (Fig. 2); however, the latter effect was modulated by the H/L ratio (incubation stage × H/L interaction: $b = 0.1$, $t = 2.87$, $df = 28$, $P = 0.01$; Table 1; Fig. 2). This interaction indicated that BKC and incubation stage had the strongest negative association in females with low H/L ratios (‘good physiological state’), but the response was dampened with increasing H/L ratios (Fig. 2) to the degree, where females with high H/L ratios (‘poor physiological state’) displayed practically no association

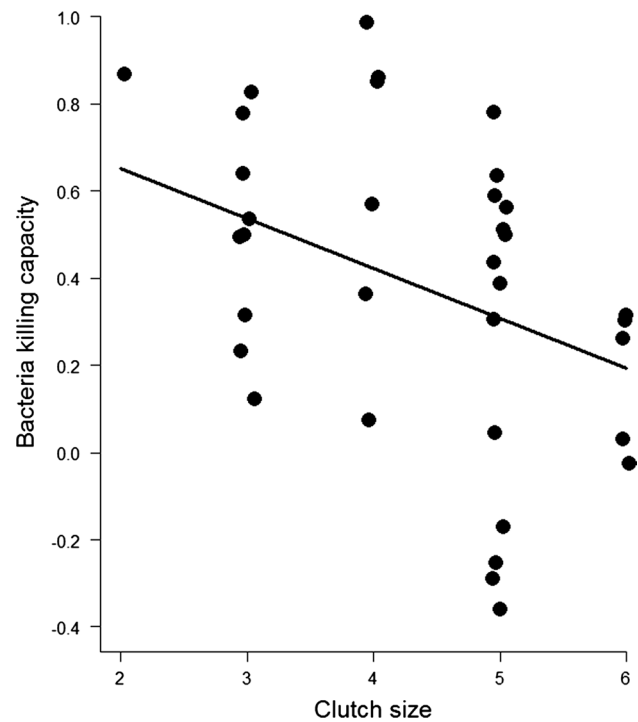


Fig. 1 Bacteria-killing capacity of blood plasma of incubating eider females is negatively correlated with clutch size

between incubation stage and the H/L ratios. In addition, as expected, BKC was generally negatively linked with increasing H/L ratios, but this effect was modulated by female breeding experience (minimum breeding experience × H/L interaction: $b = 0.09$, $t = 2.85$, $df = 28$, $P = 0.01$; Table 1; Fig. 3). This interaction showed that inexperienced females exhibited the strongest negative association between BKC and the H/L ratio, and that this effect was dampened as female minimum breeding experience increased (Fig. 3).

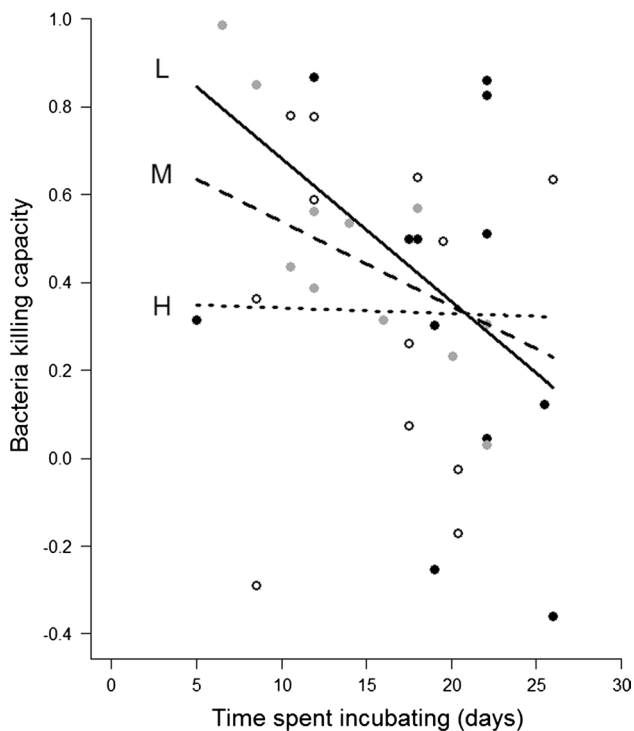


Fig. 2 Bacteria-killing capacity of blood plasma of eider females is negatively correlated with time spent incubating the clutch. This effect is, however, modulated by physiological state, so that females in a good state (*L* low H/L ratio, 25th percentile, *solid line, black dots*) show the steepest decline in BKC with incubation time, females in moderate state (*M* median H/L ratio, *dashed line, grey dots*) show an intermediate response, and the BKC of females in poorest state (*H* high H/L ratio, *dotted line, 75th percentile, open circles*) is essentially independent of incubation time

Discussion

Our finding that innate immune function in eider females was negatively associated with increasing clutch size and with progressing incubation is in line with our first prediction of a trade-off between immune function and reproductive effort. This is, to our knowledge, the first time, such a relationship has been demonstrated in a wild bird in its natural environment. We also found that individuals in a good physiological state (i.e., having the lowest H/L ratios) exhibited the steepest decline in BKC with advancing incubation, whereas the response gradually leveled off for females with higher H/L ratios. This finding is consistent with our second prediction that the resolution of the immunity-reproduction trade-off is state-dependent, so that individuals in a better physiological state can afford to suppress their immune system, so as to allow greater allocation of resources to reproduction. In this way, our results complement the evidence that individual quality is an important determinant of the ability to sustain acquired immune functions during the energetically demanding reproductive period (e.g., Ardia et al. 2003). Finally and,

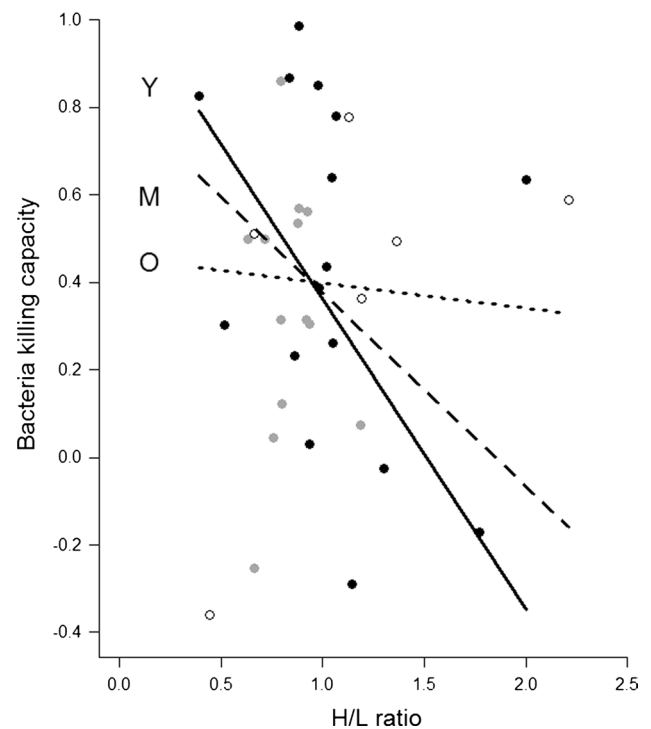


Fig. 3 Bacteria-killing capacity of blood plasma of incubating eider females is negatively correlated with the H/L ratios, a proxy of physiological state. This relationship is, however, modulated by age, so that the decline is steepest in young individuals (*Y* 25th percentile, *solid line, black dots*), becomes more gradual in females of intermediate age (*M* median, *dashed line, grey dots*), and is more gradual in older individuals (*O* 75th percentile, *dotted line, open circles*)

perhaps, intriguingly, we did not find evidence for down-regulation of immune function with increasing age (prediction 3; see “Introduction”). Instead, the H/L ratio of young females showed the strongest negative association with BKC (Fig. 3).

Potential trade-offs between reproductive investment and innate immunity

While there is a growing body of evidence, suggesting that acquired immunity and reproduction are traded off (Knowles et al. 2009), trade-offs between innate immunity and reproductive investment have rarely been demonstrated (e.g., French and Moore 2008; Ruiz et al. 2011). Thereby, the observed negative relationship between clutch size and BKC (Fig. 1) is one of the first findings consistent with a potential trade-off between innate immunity and reproductive investment, a wild bird in nature. Although little studied, such effects may be more widespread in the wild than currently appreciated (French and Moore 2008; Ruiz et al. 2011), but their detection may require special physiological conditions which apply to eiders. Eiders are open nesters with uniparental care and females depend largely on stored fat reserves during the incubation due to anorexia, which

makes total energy investment in clutch formation and incubation, especially high in this species (Thomson et al. 1998; Meijer and Drent 1999; Hobson et al. 2015). Thus, the accumulated energetic cost of a protracted incubation fast could intensify the trade-off between reproduction and immune function in eiders (Hanssen et al. 2004; Bourgeon et al. 2009), and thus make it both more detectable and more important than in other species breeding under more lenient circumstances. Along broadly similar lines, Ellis et al. (2012) found that BKC was depressed in brown-headed cowbirds (*Molothrus ater*) due to feather moult, another energy-demanding life-history stage in birds.

State-dependent decline in immunity

We predicted that individuals in a better state would be more likely to suppress their immune defences during reproduction, because they can afford to suppress their immune function to maximize reproductive success. Our results, indeed, showed that individuals in better physiological state, with low H/L ratios, exhibited higher BKC at the start of incubation as well as a marked decline in BKC with the progressing incubation stage (Fig. 2). Alternatively but not exclusively, this result could also mean that females with low H/L ratios may be challenged by the strains of breeding to the degree, where they are not able to mobilize sufficient energy to simultaneously safeguard both high reproductive performance and efficient immune defences. Thus, a previous study on eiders convincingly demonstrated that simultaneous investment into both immunity and reproduction may reduce the annual return rate of challenged individuals (Hanssen et al. 2004); however, this study did not specifically address the role of individual quality (but see Ardia et al. 2003). In contrast, females showing high H/L ratios, i.e., individuals in poor physiological state, had on average lower BKC throughout the incubation, but this low BKC was not further reduced as incubation progressed (Fig. 2). The reason for this may be that further suppression of innate immunity functions could potentially prove fatal for females in a poor state, whereas the BKC of females in a good physiological state may have been well above the minimum requirements for adequate immune defence against infection at incubation onset.

It has been proposed that individuals should not compromise their first-line defences, because of their central role in preventing pathogenic invasions prior to the triggering of the acquired immune response (e.g., Delves et al. 2011). For example, another component of innate immunity, plasma nitric oxide (NO), is not suppressed during incubation in eiders (Bourgeon et al. 2007). One possible reason for the apparent discrepancy between our results and those of Bourgeon et al. (2007) could be that eider females breeding in the high Arctic (Svalbard) may be exposed to a generally

harsher climate, which may not allow immunosuppression without detrimental effects on future survival and fecundity. However, this explanation seems less likely given that the mean H/L ratios at our study site (1.06 ± 0.04 , mean \pm SE) are at the high end of published estimates, being considerably higher than, for example, those reported from an eider population at Tromsø, northern Norway (0.44–0.65; Hanssen et al. 2003). Thus, it is conceivable that individuals in a better state may be able to compensate for the loss in BKC by the other immune defence components, such as NO. Individuals in a poor physiological state may simply lack the requisite resources to allow such compensation. Although changes in BKC over the course of incubation seem to be associated with the H/L ratio, suggesting state-dependent immunosuppression, we found no such effect of body condition on BKC. It is also pertinent in this respect that other studies have also failed to find a connection between BKC and body condition (Merrill et al. 2014; Downs et al. 2015), an open question requiring more experimental study.

Age-related change in immunity and physiological state

We found no clear-cut support for the prediction that older females would invest more into reproduction and less into self-maintenance, manifest as a general decline in BKC with advancing age. Rather, the relationship between innate immunity and age was mediated by the degree of physiological challenge (H/L ratio), such that young individuals exhibited a pronounced decline in BKC with an increasing H/L ratio, whereas this negative relationship became increasingly gradual with advancing age (Fig. 3; Table 1). Although this result seems contrary to our a priori expectations, it may actually reveal an error in the underlying assumption that reproductive value should decline with age (e.g., Bouwhuis et al. 2010, 2012) in the subset of individuals under study, or even in the study species in general. Eiders have long life expectancy (ca. 21 years) and mortality rates only begin to increase after 17 years of age (Coulson 1984), whereas only one oldest female in our sample had an estimated minimum maternal experience of 16 years (although actual age is obviously higher with age of first breeding at 2–5 years; Hario and Rintala 2009). Therefore, we may not have a sufficient representation of the very oldest age classes to be able to detect the effect of decreasing residual reproductive value on immune function. More generally, eiders, just like many species of birds, may actually show a pattern of increasing reproductive value with age, resulting from age-related improvements in reproductive success and/or survival (Proaktor et al. 2007). Thus, survival in eider females slightly increases with age when comparing juveniles, immature females, and established breeders (Kats 2007). Furthermore, fecundity (e.g., Baillie and Milne 1982; Öst and Steele 2010) and attractiveness as

partner in reproductive cooperation (Jaatinen and Öst 2011) are positively correlated with age in eiders. Assuming that young breeders may have a lower probability of returning to breed (i.e., low reproductive value), their optimal strategy may actually be to put greater emphasis on current reproduction by reducing immune investment when subjected to increased reproductive effort (i.e., increased H/L ratios) (see Ardia 2005b). On a proximate level, immune defences obviously need not be actively down-regulated in such individuals; rather, younger birds may simply be unable to maintain BKC under physiological challenge.

The lower rate of change in BKC with increasing H/L ratio in older individuals (Fig. 3) can also be explained by non-random disappearance of the lowest-quality individuals from the population (i.e., selective disappearance). An appropriate level of immune function, whether mediated by innate or acquired mechanisms, is essential for survival (Janeway et al. 2001; Møller and Saino 2004; Morgan et al. 2005). Thus, it is plausible that the weaker relationship between BKC and the H/L ratio in old individuals may be due to low-quality individuals, i.e., those with both low immune function and high H/L ratios, selectively disappearing from the population before reaching old age. In other species, high H/L ratios have been linked to increased risk of infections (Al-Murrani et al. 2002) and reduced survival prospects (Lobato et al. 2005; Kilgas et al. 2006). Furthermore, high H/L ratios are associated with poor body condition at hatching in eiders (Hanssen et al. 2003), and poor body condition at hatching is, in turn, related to lower survival, as shown in a previous study from our study population (Ekroos et al. 2012). Thus, eider females with high H/L ratios and low BKC may be underrepresented in the older age classes, which may contribute to a more gradual relationship between H/L ratio and BKC among the older surviving individuals.

Taken together, this study is one of the first to suggest that innate immunity may be depressed during reproduction in a wild bird. It also underlines the importance of physiological state and age in shaping the intricate connections between immunity and reproduction (e.g., Ardia 2005a, b; Love et al. 2008). By way of caution, it should be kept in mind that the data at hand are correlational and cross-sectional, and so do not exhibit sufficient resolution to assess individual changes in immune function. We, therefore, welcome future longitudinal research, preferably involving experimental manipulation, addressing age-dependent connections between reproduction, immunity, and physiological state. In particular, we encourage future studies investigating potential compensatory mechanisms between various components of the immune system, a research field in which the current knowledge gap is, perhaps, the most poignant. Because immune function is under strong influence by the environment, we strongly encourage such studies to be conducted in the natural environment of the study organism.

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Author contribution statement KN and KJ originally formulated the idea, SAN, MÖ, and KJ conducted fieldwork and the SAN performed laboratory analyses, KJ and KN analysed the data, all authors contributed to manuscript writing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement Eider female handling procedures were approved by the Animal Experiment Board/State Provincial Office of Southern Finland (permit number ESAVI/1697/04.10.03/2012). Female trapping procedures also complied with the regulations of the Tvärminne Zoological Station. The ethics involved in female capture are explained in more detail by Kilpi et al. (2001).

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