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RESEARCH ARTICLE

Reduced immune responsiveness contributes to winter energy conservation in an Arctic bird

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ABSTRACT

Animals in seasonal environments must prudently manage energy expenditure to survive the winter. This may be achieved through reductions in the allocation of energy for various purposes (e.g. thermoregulation, locomotion, etc.). We studied whether such trade-offs also include suppression of the innate immune response, by subjecting captive male Svalbard ptarmigan (*Lagopus muta hyperborea*) to bacterial lipopolysaccharide (LPS) during exposure to either mild temperature (0°C) or cold snaps (acute exposure to −20°C), in constant winter darkness when birds were in energy-conserving mode, and in constant daylight in spring. The innate immune response was mostly unaffected by temperature. However, energy expenditure was below baseline when birds were immune challenged in winter, but significantly above baseline in spring. This suggests that the energetic component of the innate immune response was reduced in winter, possibly contributing to energy conservation. Immunological parameters decreased (agglutination, lysis, bacteriostatic capacity) or did not change (haptoglobin/PIT54) after the challenge, and behavioural modifications (anorexia, mass loss) were lengthy (9 days). While we did not study the mechanisms explaining these weak, or slow, responses, it is tempting to speculate they may reflect the consequences of having evolved in an environment where pathogen transmission rate is presumably low for most of the year. This is an important consideration if climate change and increased exploitation of the Arctic would alter pathogen communities at a pace outwith counter-adaption in wildlife.

KEY WORDS: Acute-phase response, Arctic, Body temperature, Fever, Innate immunity, Thermoregulation

INTRODUCTION

Animals in seasonal environments face a thermal transition as summer turns to winter, with ambient temperature (T_a) often fluctuating more than 50°C between seasons. Non-migratory and non-hibernating endotherms meet this challenge by various combinations of seasonal increases in insulation and body reserves, and adequate thermophysiological responses, including thermogenesis when needed (e.g. Scholander et al., 1950a,b). This produces winter phenotypes on a continuum of scales from hypometabolic, rather inactive, well-insulated and starvation-tolerant large animals (Blix, 1989; Signer et al., 2011), to hypermetabolic and quite active smaller animals that maintain high food intake and heat production on account of comparatively poorer insulation and limited capacity to store fat (Haftorn, 1992; Swanson and Vézina, 2015). Regardless of strategies involved, it is clear that prudent energy management is key to successful endurance of long and cold winters where biomass production is low, the short photoperiod constrains the time available for foraging and snow hampers food access. Under such circumstances, wintering animals are hypothesized to prioritize resource allocation to pressing matters, viz. thermoregulation, over that to less urgent bodily demands (cf. Drent and Daan, 1980).

The potential trade-off between thermoregulation and the immune defence system is particularly interesting because, much like staying warm, the ability to keep pathogens at bay is crucial for survival but is associated with (energetic) costs (Klasing, 2004; Martin et al., 2008a; Demas et al., 2012; Hasselquist and Nilsson, 2012). For example, febrile responses elevate metabolic rate by 10–20% per °C increase in body temperature (Kluger, 1991; Marais et al., 2011a), and animals producing antibodies against novel antigens but without fever still spend more energy than when non-immunized (Demas et al., 1997; Svensson et al., 1998). By analogy, a challenge to the immune defence system may prompt 'sickness behaviours' (*sensu* Hart, 1988), i.e. behavioural modifications that reduce energy expenditure (Ilmonen et al., 2000; Råberg et al., 2000; Bonneaud et al., 2003; Nord et al., 2014). This could reflect the fact that an imbalance between resource demands for an immune response to infection and resources available to cover these costs can have serious consequences (Moret and Schmid-Hempel, 2000). For this reason, we might expect a trade-off between optimal immune responses and increased needs for cold protection under low resource availability in winter (cf. Downs et al., 2013).

An energy-based trade-off between immune function and thermoregulation could explain why some studies have found that animals mount weaker immune responses when food is limited (Demas and Nelson, 1998; Buehler et al., 2009; Cornelius Ruhs et al., 2019) (but see Schultz et al., 2017; Xu et al., 2017). Interestingly, low T_a does not seem to be immunosuppressive per se, because measurements of the energetic consequences of mounting an immune response in cold conditions reveal that metabolic costs are additive to those of thermoregulation (Burness et al., 2010; Hawley et al., 2012; King and Swanson, 2013). These studies were performed after acclimation to low T_a with food *ad libitum*. This design might be unsuitable to reveal any energy-based trade-offs because, in the wild, changes in weather can instantly alter thermoregulatory costs and food availability. Consequently, there is a need to study responses to sudden temperature drops typical for high-latitude winters, e.g. by assessing energetic investment in the immune response when facing acute changes in T_a.

The role of day length as a modulator of immune responses has been proposed to be less important than seasonal variation in body condition (Ashley and Wingfield, 2012; Carlton and Demas, 2015).

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For example, song sparrows (*Melospiza melodia morphna*) lose proportionally more body mass after an immune challenge in winter, when they are heavier, than in spring, when body mass is lower. This has been proposed to reflect seasonally varying energetic investment in immune function (Owen-Ashley and Wingfield, 2006). This is peculiar if true, because if the strength of the immune response is resource limited, it seems maladaptive to mount stronger responses during the nutritionally most constrained time of year, when larger reserves should be important to survive starvation. In line with this, skylarks (*Alauda arvensis*) do not seasonally modulate energetic investment in innate immune responses despite significant changes in fat reserves throughout the year, suggesting that adequately responding to infection is so crucial for survival that the response is not compromised (Hegemann et al., 2012). When also considering how photoperiod shapes energy budgets in diurnal animals via its influence over time for energy acquisition and length of the overnight fast, the conclusion that seasonal variation in the strength of innate immune responses is governed by variation in body condition (i.e. fat reserves) appears potentially premature. Studies of sub-polar or polar animals seem an ideal system to provide new insights into this question, because they rely extensively on accumulated fat reserves in winter (e.g. Blix, 2016) when energy cannot be easily replenished because of the very short or non-existent periods of light, sometimes combined with a photoperiodically controlled reduced appetite (Stokkan et al., 1986). By contrast, these animals enjoy analogously relaxed constraints on foraging time in spring and summer (Mortensen et al., 1983) when the sun never sets.

We studied how investment in the innate immune response changed during an acute cold challenge in winter and spring in the Svalbard ptarmigan (*Lagopus muta hyperborea* Sundevall), the world's northernmost resident bird, which is endemic to the Svalbard archipelago and Franz Josef Land (at 77–81°N). In this bird, winter acclimatization involves moult into a highly insulating winter plumage (Mortensen and Blix, 1986; Nord and Folkow, 2018), deposition of substantial fat stores (Mortensen et al., 1983) and reduced locomotor activity (Lindgård et al., 1995), all of which contribute to energy conservation at the times of year when it is dark and food is scarce. Consequently, fat reserves decline gradually during the dark winter months, even in captivity with *ad libitum* access to food, until birds are almost fat-free in late spring or early summer (Mortensen et al., 1983; Stokkan et al., 1986; Nord and Folkow, 2018). Thus, birds have larger fat reserves when the environment constrains foraging the most, but carry less fat when photoperiod makes it easier to find food. The combination of large fat reserves and continuous darkness on the one hand, and lower body reserves but continuous light on the other, provides an excellent opportunity to evaluate the roles of reserve levels and environmental constraints for investment in immune function. We therefore triggered an innate immune response using *Escherichia coli* lipopolysaccharide (LPS), a non-pathogenic antigen that typically induces a fever response largely similar to that during pathogenic infection (Ashley and Wingfield, 2012), in constant darkness in winter and in constant light in late spring, both when birds were in thermoneutral T_a (0°C) and when they were suddenly exposed to a T_a far below thermoneutrality (−20°C; to simulate a cold snap) (Mortensen and Blix, 1986; Nord and Folkow, 2018) (Fig. 1A). We then measured effects on thermal (several body temperatures), metabolic (resting metabolic rate, RMR) and immunological responses (lytic enzymes, natural antibodies, bacteriostatic capacity, concentration of the acute-phase protein haptoglobin/PIT54). We also recorded sickness behaviour (duration of anorexia, food intake) and its consequences (changes in body mass and fasting resistance) until 3 weeks after the challenge. We predicted that the energetically costlier low T_a would always suppress the innate immune response, but expected that the effect of photoperiod could take two directions: (1) larger body reserves in winter might allow higher investment than in spring, or (2) winter acclimation, involving measures that put these birds into an energy-conserving mode, may favour reduced responses in winter to safeguard fat reserves for energetic emergency.

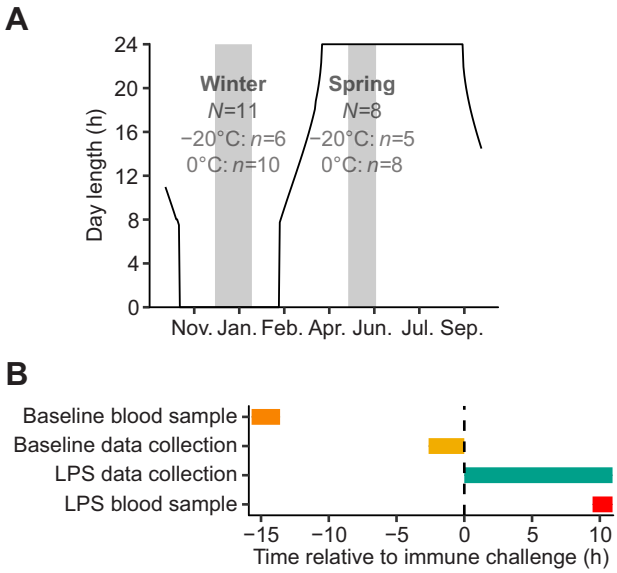

Fig. 1. Seasonal timing of the experiment and timeline of measurements. (A) Natural variation in photoperiod in Longyearbyen, Svalbard (78°13′N, 15°38′E) (which was simulated in captivity), experimental periods (grey bars) and sample sizes for each season and ambient temperature. *N* refers to the number of individuals used, whereas *n* refers to the number of measurements. (B) Timeline of the immune-challenge experiment [intramuscular lipopolysaccharide (LPS), 1 mg kg^{-1}]. A single bird was never measured more than once per ambient temperature (T_a) in each season. Seven of the 8 birds used in spring had also been measured in winter.

MATERIALS AND METHODS

Birds and housing

We used 12 physically mature (i.e. at least in their second year) male Svalbard ptarmigan, of which six were bred at UiT The Arctic University of Norway. The remaining six were captured as 1–3 week old chicks near Longyearbyen, Svalbard (78°13′N, 15°38′E), 16–17 months before the experiment started, after which they were kept and fed identically to the captive birds. Housing conditions are detailed in Nord and Folkow (2018). Morphological and physiological aspects of winter acclimation did not differ between the captive-bred and wild-caught birds [body mass gain (summer minimum until start of experiment): t_{10}=0.78; dissectible fat gain (corresponding time period): t_{10}=0.97], which is typically the case when Svalbard ptarmigan of different origin are maintained under the same photoperiod (Stokkan et al., 1986; Lindgård and Stokkan, 1989; Nord and Folkow, 2018, 2019). The study was restricted to males, because at the time of the study all females in the facility were part of the breeding population that is exempt from experiments all year.

Experimental protocol

Each bird was its own control, and was measured following the timeline in Fig. 1. Data were collected in winter (constant darkness,

DD) from 7 December 2015 to 17 January 2016 when birds had substantial fat reserves (mean±s.d. body mass: 1064.2±33.0 g, dissectible fat: 261.5±16.7 g), and in spring (constant light, LL) from 3 May to 3 June 2016 when birds were still in winter plumage but reserve levels were lower (body mass: 828.1±18.1 g, dissectible fat: 142.0±9.2 g) (Fig. 1A) (dissectible fat levels calculated according to Mortensen et al., 1983). During the 16 weeks in between these experimental periods, birds were undisturbed apart from weekly cleaning of cages and fortnightly weighing, which is part of the normal housing regime in the facility. Birds were measured at (mean±s.d. T_a=−0.1±1.6°C) and below (T_a=−19.9±0.5°C) thermoneutrality (henceforth 0°C and −20°C, respectively) (Mortensen and Blix, 1986; Nord and Folkow, 2018). We used 11 birds in winter, of which 10 were measured in 0°C and 5 in −20°C. One bird was measured only in −20°C (i.e. 0°C; n=10; −20°C: n=6). In spring, we used 8 birds (i.e. 0°C: n=8; −20°C: n=5), of which 7 had also been measured in winter (Fig. 1A). In winter, seven birds were first measured in 0°C. The remaining birds were measured first in −20°C and then in 0°C. In spring, all birds were first measured in 0°C and then in −20°C. We could not sample all individuals in both temperatures in winter because it was not always possible to accommodate the 21 days post-treatment measurements (below) within the period of constant darkness. In spring, measurement in both temperatures was not always possible as some individuals were allocated to the breeding population by the end of May.

We weighed the birds (±0.1 g) and collected a baseline blood sample (300–400 µl) from the basilic vein (using a 26-gauge needle with a 1 ml syringe) within 3 min of capture and 11 h 47 min±1 h 2 min (mean±s.d.) before LPS injection [at 22:18 h (±63 min s.d.) local Tromsø time]. The blood was kept on ice for <10 min until centrifugation (10,000 rpm, 10 min) and plasma was stored first at −80°C and then at −50°C until analysed. The following morning, 12 h 13 min±1 h 3 min after collection of the baseline sample [i.e. at 10:06 h (±31 min s.d.) local time], birds were weighed and instrumented with 36-gauge type T (copper-constantan) thermocouples (Omega Engineering, Norwalk, CT, USA) for measurement of core body temperature (T_c; 70 mm into the cloaca), back skin temperature (T_{back}; between the wings) and head skin temperature (T_{head}; on the scalp). Attachment and calibration followed Nord and Folkow (2018). All procedures in winter were performed in darkness, only under illumination from a red light head torch. Weighing and instrumentation were completed within 10 min, a time period that is typically not sufficient to affect the innate immune response (e.g. Buehler et al., 2008; Zylberberg, 2015; but see Gao et al., 2017). All but three birds had been subjected to a similar handling and measurement protocol on 6±2 (mean±s.d.) instances ranging from 4 to 13 months before the experiment, as part of a previous study (for details, see Nord and Folkow, 2018), i.e. they were habituated to the procedures.

Birds were subsequently put into a 33.6 l transparent metabolic chamber (that was ventilated with ambient air at 5.1±0.3 l min^{-1}) inside a climatic chamber (24/50 DU, Weiss Technik, Giessen, Germany) for measurement of body temperatures, and RMR using open-flow respirometry. In winter, O_2 consumption was measured using a S3-A oxygen analyser (Applied Electrochemistry, Pittsburgh, PA, USA), and CO_2 production was recorded using a ML206 gas analyser (ADInstruments, Sydney, NSW, Australia). In spring, O_2 consumption was measured using a FC-10A oxygen analyser (Sable Systems, Las Vegas, NV, USA). Flow rate was registered with a FMA-A2317 mass flow meter (Omega Engineering, Norwalk, CT, USA), and humidity and temperature of the sample gas were measured using a HMI32 thermometer and hygrometer (Vaisala, Vanda, Finland). Air temperature inside the metabolic chamber was monitored with a 20-gauge type T thermocouple (Omega Engineering) positioned in the chamber ceiling, at a height at which heat produced by the bird did not affect the reading. We calibrated the O_2 analysers against ambient air (20.95% O_2) and 100% N_2 (i.e. 0% O_2), and also using the N_2-dilution technique (Fedak et al., 1981). The CO_2 analyser was calibrated against 100% N_2 and 1% CO_2. We calibrated all analysers daily, and used day-specific calibration values to convert measured voltage to gas concentration. The FMA-A2317 mass flow meter was factory calibrated 6 months prior to use. All data were recorded and digitized from raw signals using a ML796 PowerLab/16SP A-D converter (ADInstruments). Between-instrument variation in O_2 concentration measurement was corrected using the daily N_2-dilution data following Nord and Folkow (2018). Illumination was by dim red light (≪1 lx) in winter, and full-spectrum white light in spring.

After 1 h 44 min (±13 min s.d.) equilibration, we collected baseline thermal and metabolic data for 20 min and then injected 1 mg kg^{-1} LPS (L2880; Sigma-Aldrich Norway AS, Oslo, Norway) carried in 100 µl PBS (D8662, Sigma-Aldrich) into the pectoral muscle, at a depth of 18 mm immediately lateral to the sternal keel. This, and lower, doses evoke fever/metabolic responses in several bird species (e.g. Marais et al., 2011b; Nord et al., 2013; Sköld-Chiriac et al., 2014, 2015). We then recorded body temperature and RMR at 30–40 min intervals in between 20–30 min measurement of baseline air, for 10 h 26 min (±19 min s.d.; range 9 h 21 min to 10 h 53 min). This period encompasses the majority of the expected immediate thermal/metabolic responses to LPS (e.g. Marais et al., 2011b; Nord et al., 2013; Sköld-Chiriac et al., 2015). The respiratory quotient (RQ) during these measurements was 0.70±0.04 (mean±s.d.). At the end of the metabolic/temperature measurements [i.e. at 22:39 h (±53 min s.d.) local time], we swiftly removed the thermocouples, collected a 300–400 µl post-LPS injection blood sample (from the basilic vein on the wing opposite to where we collected the baseline sample) and weighed the bird. Samples were processed identically to baseline samples. We weighed the birds daily until 7 days after LPS injection, every other day until 14 days after injection, and again at 21 days after injection. Food consumption was recorded daily during this period. For birds measured in both T_a, the second challenge was performed 27±3 days (mean±s.d.) after the first (range 24–31 days), which is well beyond the expected time frame of blunted responses to LPS from immunological learning (≤10 days; Marais et al., 2011b).

Ethics

Bird capture and import were permitted by the Governor of Svalbard (permit no. 2014/00290-2 a.522-01), the Norwegian Environment Agency (permit no. 2018/7288) and the Norwegian Food Safety Authority (permit no. 2014/150134). The Norwegian Food Safety Authority approved the experimental protocol (permit no. 6640).

Immunological measurements

All assays were performed blind with respect to season, T_a and type of sample (i.e. baseline, post-LPS challenge).

Haemolysis and haemagglutination

We used a haemolysis–haemagglutination assay that is believed to quantify lytic enzymes of the complement system (lysis) and non-specific natural antibodies (agglutination) (Matson et al., 2005). Natural antibodies are important recognition molecules that

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initiate the complement cascade, ultimately resulting in cell lysis (Caroll and Prodeus, 1998). Samples were serially diluted and incubated with a 1% rabbit red blood cell suspension (B-0009H, Harlan Laboratories, Loughborough, UK). Following incubation (90 min at 37°C), plate images were collected after 20 min (agglutination) and 90 min (lysis). Images of individual samples were randomized and scored twice, always blind with respect to sample identity [coefficient of variation (CV) (chicken standard) within/between plates: 0.03/0.11 haemolysis; 0.05/0.23 haemagglutination].

Bacteriostatic capacity

We quantified the capacity of plasma to kill or otherwise prevent replication of *E. coli* (bacteriostatic capacity; also referred to as bacteria killing ability, BKA), an integrative measure of innate immune function that includes natural antibodies, complement and phagocytosis, following French and Neuman-Lee (2012). We diluted 6 µl plasma in 4 µl 10^5 *E. coli* solution (ATCC 8739, MicLev, Malmö, Sweden). This concentration was based on preceding validation from a dilution series using blood plasma from Svalbard ptarmigan that were otherwise not part of the experiment. We then measured bacterial growth spectrophotometrically at 600 nm after 12 h incubation at 37°C (Eikenaar and Hegemann, 2016). We used four negative controls per plate. Samples were run in triplicate, and averages were used in all analyses (CV: 0.16). When one replicate was >2 s.d. from the others, we calculated averages without this observation (7 of 63 cases).

Haptoglobin/PIT54

Haptoglobin/PIT54 is synthesized by the liver during the acute-phase response, and is released into the circulation to limit the pathogen's access to iron (Cray et al., 2009). We measured the concentration of this protein using a colorimetric kit (TP801, Tri-Delta Diagnostics, Boonton, NJ, USA) with minor modifications to the manufacturer's instructions (Matson et al., 2012). We measured absorbance at three wavelengths (405, 450 and 630 nm) prior to the addition of the final reagent that initiated the colour-change reaction. We corrected for differences in plasma colour/cloudiness by subtracting pre-scan from final absorbance values. Final data were corrected for between-plate variation. Samples were run in duplicate in all but 13 (of 63) cases (CV=0.14) where the remaining plasma volumes were too low. Averages were used in all analyses. Three marginally negative values (of 112) were set to 0.

Data analyses

Missing data

Sample sizes in the final dataset differ slightly between response variables. Specifically, we excluded thermal and metabolic data from measurement periods where the birds were not at full rest, as judged from video recordings (21 of 360 cases). If a bird was not at rest during the period dedicated to baseline data collection, we used resting data from an earlier time point during the thermal equilibration period. We then dismissed data from thermocouples that fell out/off or broke, which occurred in spring only (T_c: 7, T_{back}: 0, T_{head}: 55, of 339 remaining recording periods). Two birds (spring, 0°C: 1; winter, 0°C: 1) for which rest data could only be collected for 5 h 59 min and 6 h 29 min after the immune challenge were excluded from calculation of metabolic and thermal responses.

We lost one baseline blood sample (spring, 0°C) because the tube broke during centrifugation. We assigned this bird the pre-injection immunology parameters corresponding to the global average for this season and T_a.

We excluded body mass and food consumption data from four birds that died before the end of the 21 day observation period (winter: 2, 7 and 11 days post-LPS; spring: 2, 1 day after the LPS challenge). In spring, collection of body mass and food consumption data ended 13 days after the immune challenge for two birds, when they were allocated to the onsite breeding programme. Food consumption data were not available for days 19 and 20 after the immune challenge for one bird in winter and two birds in spring because of investigator error. We assigned these days the mean of the food intake recorded on days 18 and 21. Investigator error also resulted in missing data for baseline food intake for one bird (spring, −20°C). We used food intake recorded closest in time before the immune challenge (−3 days) as the baseline for this individual.

Thermal and metabolic responses to the immune challenge

Data used for analyses were 10 min means from each measurement period. RMR (W) and whole-animal thermal conductance (C, W °C^{-1}) were calculated according to Nord and Folkow (2018).

Changes in food intake and body mass during recovery from the immune challenge

We defined 'duration of anorexia' as the time taken for food intake to return to a level higher than, or equal to, that on the day before LPS injection. The 'magnitude of anorexia' was defined as the total reduction in food intake relative to what food intake would have been had the bird maintained pre-injection consumption when anorectic:

$$M_a = 1 - \frac{\sum_1^{d_a} \mathrm{fi}_i}{\mathrm{fi}_{\text{pre-LPS}} \times d_a}, \quad (1)$$

where M_a is the magnitude of anorexia, d_a is the duration of anorexia, fi_i is food intake on day i and $\mathrm{fi}_{\text{pre-LPS}}$ is food intake on the day before LPS injection. When food intake had not recovered by the end of the 21 day study period, we set d_a to 21.

We estimated body mass on non-weighing days between 7 and 13 days post-LPS challenge by averaging between adjacent days, and between 13 and 21 days by sequentially adding average daily change to estimated body mass during the preceding day. These data were used to calculate the change in body mass during anorexia. We then estimated dissectible fat mass (Mortensen et al., 1983), and calculated the change in fasting tolerance in thermoneutrality during anorexia, assuming an energy equivalent of 31.38 kJ g^{-1} fat (Mortensen and Blix, 1985) and extrapolating pre-injection RMR at 0°C to daily energy expenditure.

Statistics

All models were run using R 3.6.1 for Windows (http://www.R-project.org/). We used linear mixed effects models (lmer, lme4 package; Bates et al., 2015) to test how mean change in absolute RMR, C and body temperatures after the immune challenge were affected by season, T_a and season×T_a. The changes in lysis, agglutination and haptoglobin were assessed in identical models. The change in bacteriostatic capacity was analysed using logistic regression with binomial error structure (glmer, lme4 package) and the fixed structure above. We tested how the immune challenge affected the duration and magnitude of anorexia (i.e. M_a), and loss of body mass and fasting resistance in the same manner. Because birds weighed less and had lower fat reserves in spring than in winter (see above), we also tested how the immune challenge affected the proportional change in body mass and fasting resistance (relative to baseline) over the anorectic period. A random intercept for bird ID

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was included in all models to deal with repeated measurements. The season×T_a interaction was removed when non-significant ($P \geq 0.05$) based on likelihood ratio tests. All other terms were retained. Multiple comparisons were performed using the emmeans package (https://CRAN.R-project.org/package=emmeans). Values in the text are predicted means±s.e.m.

RESULTS

Test statistics for all terms and parameter estimates for final models are presented in Table S1.

Metabolic and thermal responses to the immune challenge

The metabolic responses to LPS were blunted in winter compared with spring, but were not affected by T_a (Fig. 2). RMR after LPS injection decreased by 4% (from 5.62±0.25 to 5.40±0.20 W) when the birds were challenged in winter, but increased by 9% (from 6.27±0.26 to 6.84±0.26 W) in response to the same challenge in spring (Fig. 2A,B). Thermal conductance showed a similar response, decreasing by 4% (from 0.116±0.004 to 0.112±0.003 W °C^{-1}) in winter but increasing by 12% (from 0.127±0.004 to 0.142±0.005 W °C^{-1}) in spring (Fig. 2C,D). The average T_c response was negative (−0.26±0.06°C; from a baseline of 41.9 ±0.08°C) and uniform across seasons and T_a. Qualitatively, the response was triphasic, with an initial decrease of 0.69±0.06°C that occurred within 3 h of the immune challenge, followed by a subsequent increase of 0.81±0.07°C over the next 3 h, after which T_c decreased below baseline for the remainder of the session (Fig. 2E,F). The T_{back} response was negative on average, and more

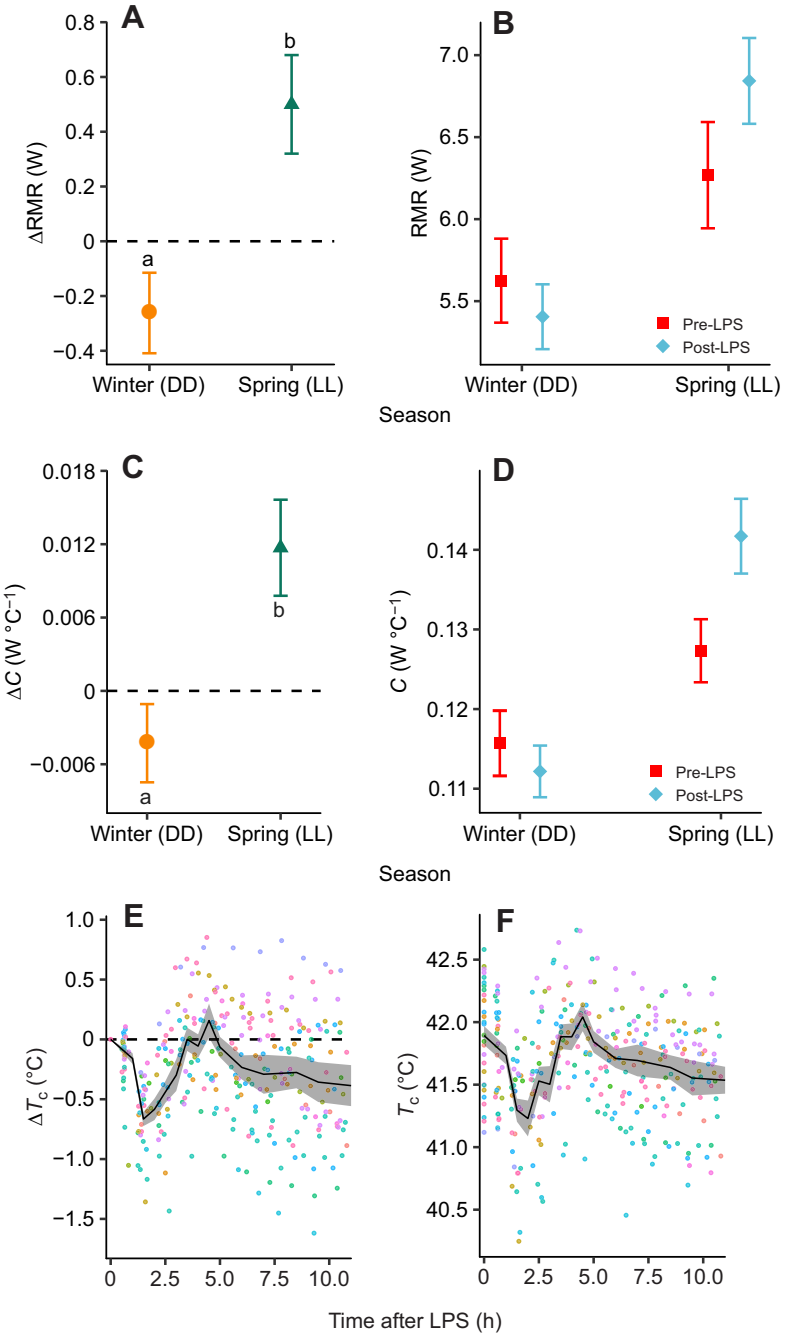

Fig. 2. Metabolic and thermal responses to an immune challenge (1 mg kg^{-1} LPS) in Svalbard ptarmigan. (A,B) Change in resting metabolic rate (RMR) from baseline (A) and absolute RMR before and after the challenge (B). (C,D) Change in thermal conductance (*C*) from baseline (C) and absolute thermal conductance before and after the challenge (D). Data in A–D are means±s.e.m. for constant darkness (DD) in winter and constant light (LL) in spring (averaged over ambient temperatures, T_a). Different lowercase letters in A and C indicate significant differences in the response. (E,F) Time course of change in core body temperature (T_c) (E) and absolute T_c (F) after the LPS challenge (averaged over T_a and seasons). The solid line and shaded areas in E and F show the averaged response±s.e.m. (based on 30 min means), and symbols are colour coded by individual. Sample sizes are reported in Materials and Methods and in Fig. 1. Statistics are reported in Table S1.

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so in spring (−0.92±0.32°C) than in winter (−0.19±0.27°C). Baseline T_{head} (32.41±0.43°C) was not affected by the immune challenge.

Immunological responses to the immune challenge

On average, the birds showed reduced lysis, agglutination and bacteriostatic capacity ca. 10 h after the immune challenge, though different parameters were differently modified by season and T_a (Fig. 3). The average reduction in lysis (0.65±0.10) differed between season and T_a combinations. Specifically, the reduction was significantly larger when birds were challenged in −20°C in winter (−40% from 3.21±0.28 to 1.92±0.08) compared with all other groups (average decrease of 22%, from 2.44±0.15 to 1.90±0.10; all $P \leq 0.05$) (Fig. 3A,B). The change in agglutination did not differ between seasons. However, it increased by 17% (from 6.46±0.55 to 7.54±0.47) after a challenge in 0°C, but decreased by 23% (from 7.18±0.73 to 5.55±0.48) after a challenge in −20°C (Fig. 3C,D). Bacteriostatic capacity decreased from 77±15% to 14±11% after the immune challenge ($P<0.001$; Fig. 3E). This response was not affected by season, T_a and season×T_a. Circulating haptoglobin (baseline: 0.12±0.02 mg ml^{-1}; response: 0.13±0.01 mg ml^{-1}) was not affected by the immune challenge (pre-LPS and post-LPS comparison: $P=0.512$).

Changes in food intake and body mass after the immune challenge

Birds reduced food intake until 9±1 days after the immune challenge, during which time they ate at 59±4% of the pre-injection level. This response was uniform in all treatment combinations. However, M_a differed by T_a. Hence, when recovering after the challenge in 0°C,

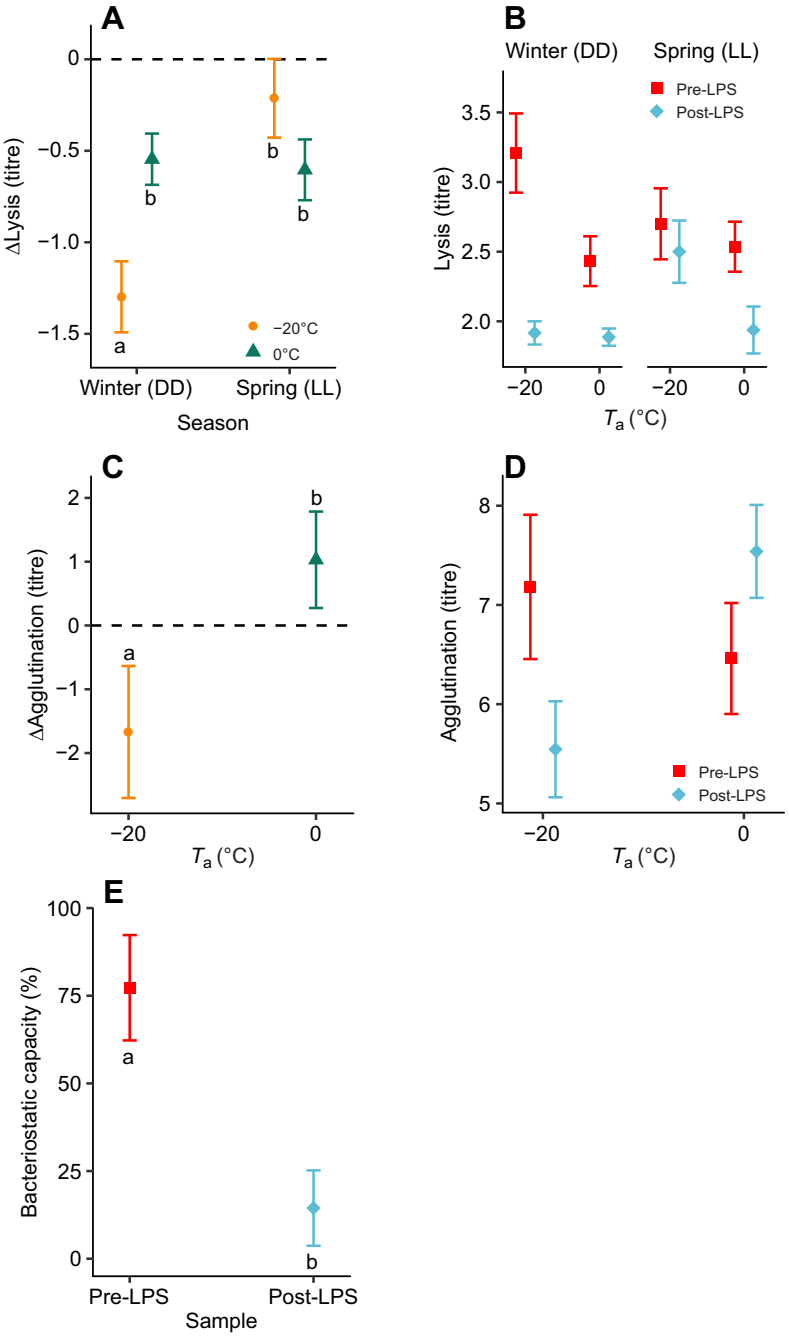

Fig. 3. Immunological responses to an immune challenge (1 mg kg^{-1} LPS) in Svalbard ptarmigan. (A,B) Change in lysis (A) and absolute lysis before and after the immune challenge (B), in a thermoneutral T_a (0°C) and after a simulated cold snap (acute exposure to −20°C), in constant darkness (DD) in winter and constant light (LL) in spring. (C,D) Change in agglutination (C) and absolute agglutination (D) in each T_a. (E) Bacteriostatic capacity of *E. coli* in blood plasma before and after the immune challenge. Grouping variables in the panels reflect final models. All data are means±s.e.m. Different lowercase letters in A, C and E denote significant differences. Sample sizes are reported in Materials and Methods and in Fig. 1. Statistics are reported in Table S1.

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birds consumed only about half the amount of food during the anorectic period compared with what they would otherwise have eaten (M_a=0.48±0.05), but intake was only reduced by ca. a third (M_a=0.34±0.06) after the challenge in −20°C (Fig. 4A). On average, body mass decreased by 77.1±10.2 g and fasting resistance dropped by 2.7±0.4 days during the anorectic period. Neither was affected by season or T_a. However, on account of seasonal changes in body and fat mass (see above), the relative effect of the immune challenge differed between seasons. Hence, body mass loss was 12±2% during the anorectic period in spring (from 799.0±30.0 to 705.9±26.1 g), but only 7±2% after the challenge in winter (from 1069.4±24.1 to 994.2 ±27.9 g). Accordingly, birds retained 83±4% of their baseline fasting tolerance in winter (15.7±0.9 days, of 18.4±0.8 days), but only 62±5% in spring (5.6±0.9 days, of 8.6±1.0 days) (Fig. 4B,C).

DISCUSSION

We show that thermoregulatory, immunological and behavioural aspects of the innate immune response in male Svalbard ptarmigan, which have evolved under extreme Arctic conditions, are modulated by the environment in complex and independent ways. Some responses changed between seasons (RMR, C, T_{back}, relative change in fasting tolerance and body mass), some were affected by T_a (lysis and agglutination, M_a), some were generally affected by the immune challenge (T_c, bacteriostatic capacity) and others showed no response to LPS (T_{head}, haptoglobin/PIT54). We discuss these findings in turn below.

Thermal and metabolic responses to the immune challenge

Lower energy costs after LPS injection when body mass peaked in winter contrasts the idea that the strength of the innate immune response is related to body reserves (e.g. Owen-Ashley and Wingfield, 2006; Ashley and Wingfield, 2012; Carlton and Demas, 2015), but conforms with the notion that winter acclimatization in Svalbard ptarmigan introduces a state of general energy conservation (Blix, 1989). In contrast, birds inhabiting areas where winter days are long enough to sustain increased energy demands could be expected to invest in the innate immune response according to current nutritional state. This might explain why the immune challenge in our study caused elevated metabolic costs (higher RMR and, consequently, C) under constant light in spring. Hence, by studying a polar animal that shows substantial natural variation in fat reserves, and lives in an environment where photoperiod varies 24 h over the course of the year, we may have revealed a response that experiments on lower latitude animals would miss.

Even when metabolic rate increased, the immune challenge provoked hypothermia (Fig. 2E,F). This is in stark contrast to the fever responses that have been recorded in birds of comparable body size injected with similar, or higher, LPS doses (cf. table 2 in Sköld-Chiriac et al., 2015). Hypothermia could be explained if the T_c prompting optimal immune function is lower than daytime resting levels but higher than night-time levels, as has been suggested for small passerines (Nord et al., 2013; Sköld-Chiriac et al., 2015). However, this does not fit with robust fever responses in other gallinaceous birds (Koutsos and Klasing, 2001). Moreover, it could be argued that the lengthy periods of body mass loss and anorexia, severalfold longer than those of other bird species (discussed below), do not corroborate the idea that the birds regulated optimal T_c during infection. Measurement of dose–responses would shed light on whether hypothermia is the typical response to infection or a consequence of severe endotoxaemia, whereas measurement in different light and dark cycles (when birds show circadian variation in T_c; Appenroth, 2016) could inform on whether the T_c response to LPS converges in days and nights in line with the hypothesis of an optimal infected T_c.

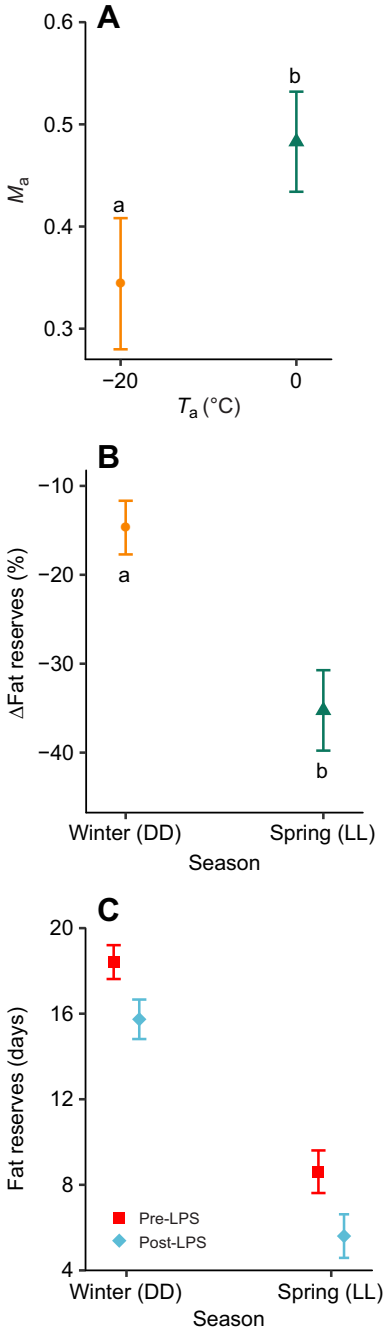

Fig. 4. Effects of an immune challenge (LPS, 1 mg kg^{-1}) on anorexia and fasting resistance in Svalbard ptarmigan. (A) Magnitude of anorexia (M_a) after an immune challenge during a simulated cold snap (acute exposure to −20°C) and after the same challenge in thermoneutrality (0°C). Birds challenged in −20°C retained ca. 65% of pre-injection food consumption, whereas birds challenged in 0°C retained only ca. 50%. (B,C) Change in estimated fasting tolerance (as fat reserves) relative to baseline, and absolute estimated fasting tolerance before and after the immune challenge, in constant darkness (DD) in winter and constant light (LL) in spring, assuming that 1 g fat contains 31.38 kJ and that baseline RMR was maintained throughout the experiment. Different grouping variables in the panels reflect final models. All data are means±s.e.m. Different lowercase letters in A and B indicate significant differences. Sample sizes are reported in Materials and Methods and in Fig. 1. Statistics are reported in Table S1.

In mammals, endotoxin hypothermia is recorded when animals are challenged in environments where energy costs of fever might be unbearable, such as during cold exposure or in neonates with poorly developed insulation and resultant high heat loss rate, and during severe infection (Jones et al., 1983; Romanovsky et al., 2005; Rudaya et al., 2005). We consider it unlikely that the T_c response was caused by energy shortage, as the birds always had ample fat reserves to fuel metabolism and never experienced particularly challenging T_a (for this species). Hence, hypothermia was perhaps an adaptive response to minimize the consequences of endotoxaemia for systemic integrity (Romanovsky et al., 1997; Liu et al., 2012). For example, it is conceivable that a fever response would have been associated with higher energy costs (e.g. Marais et al., 2011b). This would not only have required additional fat reserves but potentially also have caused increased oxidative stress (Monaghan et al., 2009). Because an immune challenge in itself can also increase oxidative stress (von Schantz et al., 1999; Armour et al., 2020), a blunted T_c response might protect the body, in line with a trade-off between immune function and oxidative stress under demanding circumstances (Eikenaar et al., 2018).

Immunological responses to the immune challenge

Other studies of birds have shown that plasma haptoglobin, lysis and bacteriostatic capacity typically increase in response to LPS (Buehler et al., 2009; Matson et al., 2012; Hegemann et al., 2013; Schultz et al., 2017). This is to be expected, given that a pathogenic insult should trigger the increased production/secretion of acute phase proteins (including haptoglobin/PIT54), the rise of some complement components and, in turn, a higher bacteriostatic capacity in the bloodstream (Leshchinsky and Klasing, 2001). In our study, the immune challenge brought general decreases to three markers of the innate immune system and did not affect a fourth (i.e. haptoglobin/PIT54). When also considering the somewhat ambiguous thermal and metabolic responses, it seems reasonable to conclude that Svalbard ptarmigan mount a different acute phase response to LPS than do other birds. This indicates that LPS had to be cleared mostly using immune cells already present in the circulation during the initial stages, resulting in decreased immune parameters 10 h after the immune challenge, while *de novo* synthesis of immune cells was either very slow or commenced later. This could also explain why haptoglobin/PIT54 levels did not increase after the immune challenge, which would have been expected over the time passed between the challenge and blood sampling (e.g. Quaye, 2008; van de Crommenacker et al., 2010; but see Hegemann et al., 2013) unless all induced synthesis was immediately utilized. Future studies should assess development of immune response markers at several time points after the challenge. At any rate, the innate immune response in this High Arctic species seems to follow an altogether different time curve compared with that of temperate (passerine) birds, which is supported by the lengthy expression of 'sickness behaviours' (anorexia and body mass loss, as discussed below). This means that recovery from the acute phase response (at least in some taxa) may be lengthier, and consequently more resource demanding, than currently assumed. Similar conclusions were recently reached in a study on fish, based on prolonged and slow changes in innate immune parameters (≥7 days) in response to an immune challenge with LPS (Vinterstare et al., 2019).

Agglutination increased slightly when birds were challenged in the mild T_a but decreased after a challenge in the cold (Fig. 3C,D). This could indicate that some aspects of the innate immune response follow a different pathway depending on the environment in which the bird contracts disease. It is also interesting to note that reduced agglutination in response to a LPS challenge (at least in −20°C) suggests that natural antibodies might be involved in ridding endotoxin from the circulation. This has been suggested before (e.g. Reid et al., 1997), but it seems more common that natural antibody concentration is not affected by LPS (Baumgarth et al., 1999; Hegemann et al., 2013, 2018; Schultz et al., 2017). Perhaps natural antibodies are significantly recruited only in particularly stressful situations, such as during severe infection or when overall immune responsiveness is low; criteria that seemed to be fulfilled by Svalbard ptarmigan in this study.

Changes in food intake and body mass after the immune challenge

Anorexia during infection (Fig. 4A) is thought to be a mechanism by which the host limits pathogen access to micronutrients, at the same time as reduced time searching for food also lowers overall energy requirements and risk of aggressive interspecific and intraspecific interactions (Murray and Murray, 1977; Hart, 1988). The period of reduced food intake in Svalbard ptarmigan surpassed the time frame reported in other (smaller) birds by about a week (Sköld-Chiriac et al., 2014, 2015), possibly as a consequence of the greater baseline fasting tolerance in larger animals. Even so, the time required to regain energy intake supports the idea that the innate immune response in Svalbard ptarmigan is slower and/or longer lasting than in temperate animals. It also suggests that Svalbard ptarmigan are more strongly affected by LPS compared with other (temperate) species, because stronger infection is normally associated with more pronounced behavioural changes during recovery (Sköld-Chiriac et al., 2015). Our data indicate that innate immune responses alone, even in the absence of replication by a live pathogen, can have much larger and longer-lasting consequences than previously assumed (see also discussion in Vinterstare et al., 2019).

The magnitude of anorexia was less when birds were challenged in the cold T_a (Fig. 4A). As for the agglutination results (Fig. 3C,D), this is consistent with the idea of immunosuppression in energetically demanding situations to promote faster, or less costly, recovery (e.g. Nelson, 2004). The response is also expected, because prolonged or deepened anorexia in the cold probably comes at a greater expense of body reserves. However, it is interesting to note that these effects were in response to acute temperature manipulation. Thus, acclimation of (some aspects of) the innate immune response was immediate and long-lasting, as the effect of a sudden drop in T_a on the recovery period was evident despite birds being returned to higher (thermoneutral) T_a after the expected time frame of the acute phase response. Future studies should attempt to elucidate the signalling mechanisms involved in mediating this response.

Birds lost more body mass in spring, possibly because higher energy costs of the immune challenge at this time of the year remained, or carried over to, recovery-phase energy expenditure. Naturally occurring higher resting energy expenditure, increased locomotor activity and faster depletion of fat reserves at this time of the year (Mortensen and Blix, 1986; Nord and Folkow, 2018) would have exacerbated this by taking a larger toll on body condition when food intake was depressed. This carried over to fasting tolerance which, given the already lower fat reserves, was only about 60% of pre-injection levels and considerably lower in absolute terms when birds had recovered from the anorexia in spring (Fig. 4A,B). Thus, while our study indicates that energetic investment in the innate immune response might be reduced in midwinter, when constant darkness combined with reduced appetite constrains refuelling,

higher investment in spring could be a risky strategy in view of lower baseline fasting resistance and faster reserve depletion rate during recovery (Fig. 4B,C). This is an important consideration for animals in the High Arctic, where lengthy spells of inclement weather are part of daily life for most of the year.

Conclusions

By acutely manipulating the thermal environment when infection hits, at times of the year with marked differences in fat reserves and ease of resource acquisition, we show that different aspects of the innate immune response in male Svalbard ptarmigan vary both seasonally and in relation to the acute harshness of the surroundings. Stronger innate immune responses in more benign environments (at thermoneutral T_a) were expected, but our data suggest this might be a risky strategy when favourable conditions are associated with a reduced safety margin (e.g. fasting tolerance). In addition, an immune challenge in spring may bring 'lost-opportunity costs' to males, if the infected animal is unable to defend its territory and partner. To this end, our study highlights how simultaneous assessment of multiple aspects of the immune response can reveal a level of complexity that analysis of independent traits may miss.

The acute phase response differed from that of other species, indicating slower innate immune responses and/or that Svalbard ptarmigan are more strongly affected by endotoxin compared with other birds. This might be a consequence of the barren environment in which these birds have evolved, where the presumably low pathogen diversity and/or transmission rates might have selected for low immune function (cf. Martin et al., 2008b). Future studies should inform this hypothesis by documenting seasonal variation and population-level effects of pathogen/parasite prevalence and/or intensity in wild Svalbard ptarmigan, as has been done for other ptarmigan species/subspecies residing at lower latitudes (Hudson et al., 1998; Holmstad et al., 2005a,b; Stenkewitz et al., 2016). Even so, our study adds to the body of evidence suggesting that Arctic wildlife might be particularly at risk when climate change alters pathogen assemblages and host–parasite interactions (Kutz et al., 2009; Altizer et al., 2013). These novel challenges to the immune defence system could be exacerbated by rapidly increasing visitor numbers to previously untouched Arctic biomes (e.g. Eeg-Henriksen and Sjømæling, 2016) and changes to phenology of migrating bird populations (Lameris et al., 2018), both of which might be agents of pathogen spread in polar regions (e.g. Olsen et al., 1996; Griekspoor et al., 2009; but see Bonnedahl et al., 2005).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.N., L.P.F.; Methodology: A.N., A.H., L.P.F.; Software: A.N.; Validation: A.N., L.P.F.; Formal analysis: A.N.; Investigation: A.N., A.H., L.P.F.; Resources: A.N.; Data curation: A.N.; Writing - original draft: A.N.; Writing - review & editing: A.H., L.P.F.; Visualization: A.N.; Project administration: A.N.; Funding acquisition: A.N.

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Data availability

Data are available from the figshare digital repository: https://doi.org/10.6084/m9.figshare.11968503.v1

Supplementary information

Supplementary information available online at http://jeb.biologists.org/lookup/doi/10.1242/jeb.219287.supplemental

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