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Article

Effects of fleas on nest success of Arctic barnacle geese: experimentally testing the mechanism

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Parasites have detrimental effects on their hosts' fitness. Therefore, behavioural adaptations have evolved to avoid parasites or, when an individual is already in contact with a parasite, prevent or minimize infections. Such anti-parasite behaviours can be very effective, but can also be costly for the host. Specifically, ectoparasites can elicit strong host anti-parasite behaviours and interactions between fleas (Siphonaptera) and their hosts are one of the best studied. In altricial bird species, nest fleas can negatively affect both parent and offspring fitness components. However, knowledge on the effects of fleas on precocial bird species is scarce. Research on geese in the Canadian Arctic indicated that fleas have a negative impact on reproductive success. One possible hypothesis is that fleas may affect female incubation behaviour. Breeding females with many fleas in their nest may increase the frequency and/or duration of incubation breaks and could even totally desert their nest. The aim of our study was to 1) determine if a similar negative relationship existed between flea abundance and reproductive success in our study colony of Arctic breeding barnacle geese *Branta leucopsis* and 2) experimentally quantify if such effects could be explained by a negative effect of nest fleas on female behaviour. We compared host anti-parasite and incubation behaviour between experimentally flea-reduced and control nests using wildlife cameras and temperature loggers. We found that flea abundance was negatively associated with hatching success. We found little experimental support, however, for changes in behaviour of the breeding female as a possible mechanism to explain this effect.

Keywords: Arctic goose colony, insect harassment, parasite–host interaction

Introduction

Parasites generally have detrimental effects on their hosts' fitness. Therefore, hosts have evolved a wide range of physiological and behavioural responses to reduce parasitic costs (Norris 2000, Clayton et al. 2010, Owen et al. 2010). Behavioural adaptations aid to avoid parasitic infection or, when already in contact with a parasite, prevent or minimize infections (Hart 1992, 1994). As a result, anti-parasite behaviours can be roughly divided into pre-infection and post-infection behaviours (Schmid-Hempel 2011). Pre-infection behaviours include temporal and spatial avoidance of parasite

prone environments, foods and individuals or hosts altering their niche to discourage parasites (e.g. prophylactic self-medication: Castella et al. 2008; see review by Curtis 2014), while post-infection behaviours consist of for example parasite removal (e.g. grooming/preening: Hart 1992, Clayton et al. 2010), and therapeutic self-medication (Clayton and Wolfe 1993, de Roode et al. 2013).

Anti-parasite behaviours can be very effective in minimizing infection risk as is exemplified by an experimental study by Daly and Johnson (2011). They showed that Pacific chorus frog larvae *Pseudacris regilla* that were anesthetized and therefore behaviourally impaired were more likely to become infected and had higher infection intensities with pathogenic trematodes (*Ribeiroia* and *Echinostoma*) than frog larvae that could display their natural avoidance behaviour. Also, when parasite removal behaviours were restrained in different animal species, ectoparasite infection increased in comparison to control animals that could perform their natural parasite removal behaviours (e.g. birds: Brown 1972, Clayton et al. 2005, Waite et al. 2012; mammals: Bennett 1969, Mooring et al. 1996).

On the other hand, such behavioural adaptations can also present fitness costs themselves. Avoidance behaviours can impose trade-offs for the host in terms of 1) decreased time spend feeding and resting, while increasing active behaviours to avoid parasites (reindeer *Rangifer tarandus tarandus*: Hagemoen and Reimers 2002, Weladji et al. 2006), 2) selection of lower quality forage over high quality parasite infested forage (sheep: Hutchings et al. 2000, 2002) and 3) increased nest desertion in the face of high nest parasite presence (e.g. cliff swallow *Hirundo pyrrhonota*: Emlen 1986; great tits *Parus major*: Oppliger et al. 1993). Furthermore, a post-infection behaviour such as parasite removal can be costly as well: it can e.g. increase energy expenditure (greater mouse-eared bats *Myotis myotis*: Giorgi et al. 2001), damage fur (moose *Alces alces*: Samuel 1991, Mooring and Samuel 1998) and decrease the available time for other behaviours such as vigilance (impala *Aepyceros melampus*: Mooring and Hart 1995).

The interactions between fleas (order Siphonaptera) and their hosts are one of the best studied (Rothschild and Clay 1952, Proctor and Owens 2000, Krasnov 2008). Fleas are typical ectoparasites of higher vertebrates and are obligatory blood-feeding insects. For the majority of flea species, adults live in a close, but temporary association with their hosts (Krasnov 2008). Their behaviour, morphology and physiology are adapted as such that they can make optimal use of their hosts temporary visits to lair, nest, dwelling or burrow (Wall and Shearer 1997). In birds, most studies on interactions between fleas and their hosts have investigated altricial species where fleas can affect both parent and offspring simultaneously and thereby decrease fitness (Richner et al. 1993, Nilsson 2003). However, knowledge on effects of nest fleas on precocial bird species, where the young only stay a short period in the nest after hatch, is scarce. Nevertheless, Harriman and Alisauskas (2010) found that nest flea abundance was negatively correlated with nesting success in precocial Ross's *Anser rossii* and lesser snow geese *Anser caerulescens caerulescens*. A possible hypothesis explaining this finding is that nest fleas affect female behaviour (Harriman and Alisauskas 2010). Alterations in female behaviour can become visible in the form of increased grooming and nest sanitation behaviours in parasite infested nests (Cantarero et al. 2013). Increased irritation might lead to higher nest desertion (Fitze et al. 2004) or possibly increases the frequency and/or duration of incubation breaks (Cantarero et al. 2013). The latter can decrease nest success when eggs get too cold and the embryo dies (Webb 1987) or when eggs are eaten by a predator during female absence (Prop et al. 1984, Samelius and Alisauskas 2001). To investigate if fleas indeed affect behaviour, experimental studies are vital to independently quantify effects of flea infestation from potential differences in the quality of the breeding individuals, which can influence their suitability and attractiveness for parasites (Krasnov et al. 2005). However, experimental studies investigating the possible mechanisms behind the failure of nests of precocial species with high ectoparasite infestation are lacking.

Here, we aim to experimentally quantify the effect of nest fleas on the behaviour and reproductive success of a precocial species; Arctic breeding barnacle geese. In barnacle goose nests on Spitsbergen (Svalbard), fleas *Ceratophyllus vagabundus vagabundus* are the only ectoparasites detected and they can be present in high numbers (Pilskog et al. 2014). In our study, we examined whether flea abundance also negatively correlates with reproductive success in this Arctic goose species. Furthermore, to investigate the hypothesis raised by Harriman and Alikauskas (2010) that fleas change female incubation and anti-parasite behaviours, we experimentally decreased flea abundance by heat treatment of nests. We compared behaviour of hosts with control and flea-reduced nests using automatically triggered wildlife cameras and temperature loggers (Cantarero et al. 2013). Our aims were to explore the changes in the frequency of anti-parasite behaviours and the frequency and length of incubation breaks as a consequence of experimentally changed nest flea abundance, and examine the possible effects of these changes on reproductive success. We expected that female geese with flea infested control nests would show increased anti-parasite behaviours, an increased frequency and/or length of incubation breaks and a lower reproductive success.

Methods

Study site and study species

We conducted this study on the islands Storholmen (ca 30 ha) and Prins Heinrichøya (ca 3 ha) in Kongsfjorden, near the village of Ny-Ålesund (78°55′N, 11°56′E), Spitsbergen (Svalbard). Since the first barnacle goose nest was detected in Kongsfjorden in the early 1980s, there has been a strong

increase in number of nests on all islands in the fjord (Tombre et al. 1998).

Observational data on flea abundance and nest success

Egg blood coverage and flea abundance

In the years 2012–2016 we took photographs of eggs in barnacle goose nests on the islands Storholmen and Prins Heinrichøya to estimate flea abundance by investigating the blood coverage on the eggs. We took one photograph per nest and from this photograph we visually estimated blood coverage of eggs on a scale from 0 to 4, with 0 being no blood at all and 4 being fully covered in blood (method adapted from Harriman et al. 2008). As eggs in the same clutch usually had similar blood coverage, the score was averaged over the nest (Harriman et al. 2008). Scoring was done by one person (MDJ) and reference photographs were used to aid in scoring.

To assess flea abundance, we collected nest material from incubating geese by quickly reaching down in the nest next to the nest cup with a hand lined by a plastic bag, to prevent fleas from escaping. We reached right up to the bottom of the nest and collected about a similar handful of nest material from all nests. The plastic bag with the nest material was closed and stored in a refrigerator at ca +5°C until extraction (for comparable methods see Pilskog et al. 2014). Extraction was done using a Tullgren funnel setup, comprised of stainless steel funnels, light fixtures consisting out of aluminum hoods (Heat reflector OLBA complete) and 25–60 W lamps (due to lamp breakages and therefore shortages Wattages differed between funnels, but were compensated for by hanging the lamp lower or higher above the sample to create a similar amount of heat). Adult fleas and larvae were forced down in the funnel because of the heat gradient and were collected in 96% ethanol in tubes at the bottom end of the funnel. Samples were extracted for approximately 48 h until dry and no fleas or larvae were seen in the material. Nest material samples were collected after extraction and dry mass of the samples was measured.

Harriman et al. (2008) found that flea numbers in the nests were positively related to blood coverage on eggs. To check whether this was also the case in our study population, we collected nest material from a subset of 109 goose nests during the breeding seasons of 2015 and 2016 to extract adult fleas and flea larvae. We also detected a positive correlation between blood coverage and adult flea numbers (generalized linear model: intercept = 3.732 ± 0.249, β blood coverage = 0.383 ± 0.148, $F_{1,107} = 6.707$, $p = 0.01$) and flea larvae numbers (generalized linear model: intercept = 4.212 ± 0.622, β blood coverage = 0.397 ± 0.193, $F_{1,107} = 4.395$, $p = 0.038$) when corrected for year variation.

Standard measurements of nest success

In the years 2012–2016, we checked all nests approximately every other day during incubation and around hatch to determine clutch size and number of hatchlings when present, and to estimate hatch date and hatching success. Clutch size was determined as the maximum number of eggs in the nest during at least two subsequent visits. When present, we defined the number of hatchlings as all eggs that showed signs of hatching and/or all successfully hatched goslings, with the assumption that all hatching eggs would become hatchlings. The number of hatchlings was counted when 1) at least half of the eggs in the nest were hatching (cracks, hatching or hatched goslings), or 2) less than half of the eggs were successfully hatched (goslings are present) while other eggs were not yet in the process of hatching. Hatching success (0 = no hatch, 1 = successful hatch) was estimated by the observation of hatching eggs, goslings or the presence of eggshells in combination with egg membranes (Davis et al. 1998). A nest was considered as successfully hatched when at least one egg had hatched. Hatch dates were estimated on the basis of signs of hatching. When at least 1 egg showed cracks the hatch date was assumed to be the day of observation plus 1 (the nest would hatch the following day). When at least 1 egg with holes, hatching goslings or still wet goslings were observed, the hatch date was the day of observation. When goslings where dry and fluffy or when we found an empty nests with eggshells and egg membranes, hatching date was assumed to be the day of observation minus 1 (the nest hatched the previous day). In the event that the islands could not be reached for multiple days in a row, because of e.g. adverse weather conditions or polar bear presence, hatch dates could not be accurately estimated and were not used. When we found an empty nest with cold eggs, only eggshells or a totally empty nest without any eggshells and membranes, this indicated that the nest was abandoned in the first case or predated in the latter two cases. Total nest predation was not taken into account as in these cases we could not distinguish whether eggs were predated because of temporal goose absence or total nest abandonment. Therefore, these nests were all grouped as unsuccessful. Partial egg predation during incubation was noted when eggs went missing in between checks or when egg predation was observed.

Experiment to reduce flea numbers

Experimental setup

In the breeding season of 2016, we aimed to reduce flea numbers by a method that has been used often to study the interaction between nest fleas and passerine birds, namely by microwaving the nest (Richner et al. 1993, Gallizzi et al. 2008). Goose nests were selected for the experiment from nests on the island of Storholmen and paired on the basis of clutch size and blood coverage (see above: blood score 1: n = 7 pairs, blood score 2: n = 7 pairs, blood score 3: n = 11 pairs, blood score 4: n = 5 pairs). Nests within a pair were randomly assigned to either a control group (n = 30) or a flea-reduced group (n = 30). On a single day (12 June 2016) all these nests were visited. Firstly, a nest material sample was taken to estimate pre-experimental flea abundance, after which the eggs were taken out of the nest and placed into a padded box. Then, all nest material was taken from the ground and placed

into a plastic bag. Nest debris from underneath the nest was also taken out as much as possible and put into a separate plastic bag. The padded box with the eggs was left near the nest location and the nest material was carried away to be treated. Fleas were eliminated from the flea-reduced nests to be by microwaving the nest material and nest debris in the plastic bag for 3 min at 900 W. Afterwards, the nest material needed to cool down before the nest was restored. The microwave appliance was fed by a relatively silent portable 230-V generator. Control nests were not treated in the microwave, but were disturbed in a similar way. All nests were regularly checked after the experiment (see above). For two nests, the eggs had been placed back in nests that turned out later to have been too warm after returning from the microwave (this was visible from burn marks on the eggs during later checks). Both nests were taken out of all analyses of data gathered after the experiment.

As nest mass and flea numbers can be positively correlated (Eeva et al. 1994, Heeb et al. 1996), we determined fresh mass of the entire nest before the experimental procedure using a digital kitchen scale positioned on a flat surface. Fresh mass of the entire nest did not differ between the experimental groups (ANOVA: $F_{1,59} = 0.089$, $p = 0.765$) and was on average 261.6 g (SD = 76.4). Also, the mass of the nest material samples did not differ between the experimental groups ($F_{1,179} = 0.123$, $p = 0.726$) and was on average 39.5 g dry mass (SD = 16.5, n = 180) or 54.5 g fresh mass (SD = 28.3, n = 165). Nest material samples were taken just before, 2 d after the experiment and 24 d after the experiment, when the eggs hatched and the geese had left.

Our experiment was effective in reducing adult flea numbers in the heat-treated nests (treatment × moment of sampling: $\chi^2_{2,8} = 13.995$, $p < 0.001$; Fig. 1A shows numbers 100 g^{-1} nest material. See below for detailed statistical methods). Before the experiment, there were slightly more fleas in control nests but this difference was not significant (post-hoc comparison; contrast control – flea-reduced: $\beta = 0.646 \pm 0.347$, z ratio = 1.857, $p = 0.063$). Two days after the experiment however, the heat-treated group had significantly less fleas than the control group (post-hoc comparison; contrast control – flea-reduced: $\beta = 2.164 \pm 0.416$, z ratio = 5.205, $p < 0.001$) and this difference was more pronounced 24 d after the experiment (post-hoc comparison; contrast control – flea-reduced: $\beta = 2.195 \pm 0.332$, z ratio = 6.616, $p < 0.001$). Heat-treatment of nests also decreased flea larvae numbers (treatment × moment of sampling: $\chi^2_{2,8} = 19.285$, $p < 0.001$; Fig. 1B shows numbers 100 g^{-1} nest material). Before the experiment, there was no difference in the number of flea larvae between the groups (post-hoc comparison; contrast [control – flea-reduced]: $\beta = 0.035 \pm 0.405$, z ratio = 0.086, $p = 0.932$). Two days after the experiment, larvae numbers decreased in both groups, but there were significantly less larvae in the heat-treated group (post-hoc comparison; contrast [control – flea-reduced]: $\beta = 2.548 \pm 0.428$, z ratio = 5.945, $p < 0.001$). This difference was still visible 24 d after the experiment

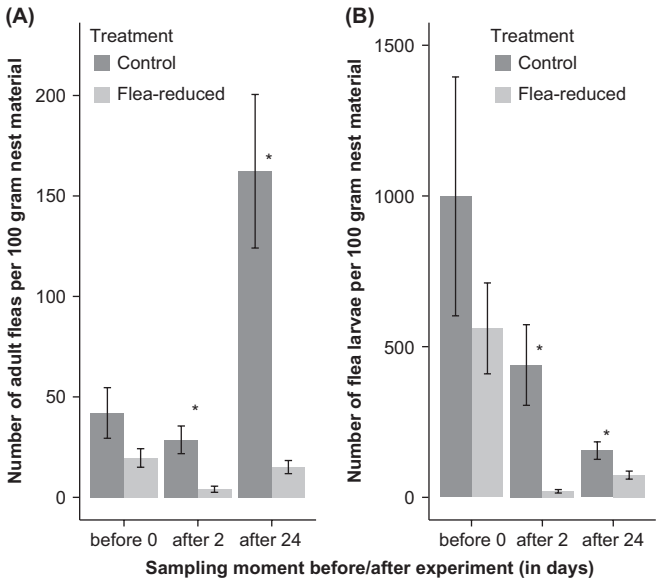

Figure 1. Differences between control nests (control, n = 30) and heat-treated nests (flea-reduced, n = 30) in (A) the number adult fleas 100 g^{-1} nest material and (B) the number of flea larvae 100 g^{-1} nest material sampled before the experiment (0 d, sample before experiment on the same day) and after the experiment (2 d and 24 d after). Error bars depict standard error.

(post-hoc comparison; contrast [control – flea-reduced]: $\beta = 0.999 \pm 0.408$, z ratio = 2.446, $p = 0.014$).

Nest temperature data

We placed temperature loggers (DS1921G-F5 thermochron iButton device, Maxim Integrated) in a subset of nests (n = 56) to investigate the effects of fleas on nest temperature and nest temperature fluctuations. The iButton loggers were glued on top of 70 mm golf tees and pushed into the nest material in the centre of the nest in such a way that the logger rested on top of the nest material and was in contact with the eggs (Ringelman and Stupaczuk 2013). The iButton loggers recorded nest temperature on three dates for which a full day was recorded with a temperature measurement every minute (before the experiment on 10 June 2016, and after the experiment on 15 June 2016 and 21 June 2016). Unfortunately, the iButtons were apparently too low and too insulated in the nest and we were therefore unable to extract accurate data on incubation recesses as in Ringelman and Stupaczuk (2013). So the data were not suitable for determination of fine-scale presence/absence, but we deemed it possible to gain information on crude scale absence/presence by investigating overall nest temperature and nest temperature fluctuations. We expected and observed on basis of visual inspection of the data that when a goose left the nest for a longer time period, nest temperature would still drop, but more slowly. We therefore calculated daily average temperature per nest and, as a measure of daily temperature fluctuation, we calculated the standard variation per nest. We compared these measures between the experimental groups to gain insight in

whether fleas affect goose incubation behaviour. To investigate the frequency of absence (goose was not sitting on nest) and absence time, we used data collected by the wildlife cameras (see below).

Wildlife camera observations

We placed in total 20 wildlife cameras (Maginon WK3 HD) at a distance of 1 m from a random selection of flea-reduced (n = 10) and control nests (n = 10) to study female incubation and anti-parasite behaviours. The camera was set to take a photograph every time the goose moved with a minimum interval of 5 min in between photos. We scored goose behaviour from photos taken on days when 1) geese were not disturbed by researchers, 2) this day was at least one day before hatching (seen from goose posture, presence of eggshells/goslings) and 3) all cameras were working properly (i.e. on 1, 2 d before and 1, 3, 4, 5, 7, 10, 12 d after the experiment). On average, 80.3 (SD = 56.9) photos were taken of a nest per day. We used an ethogram to aid in scoring of behaviour and scoring was done by three observers who were blind to treatment group. Photos that were of such bad quality that nothing could be seen (e.g. sun glare), were taken out of the analyses. We scored all photos where a goose was sitting on her nest (assuming incubation: presence) as 1 and when a goose was not incubating (i.e. standing on the nest/leaving nest/arriving at nest/absent from nest: absence) as 0. First, we were interested in the frequency and time of absence and therefore we used the time stamp of the photos to calculate the time between all consecutive photos of nests. We then selected all photos on which the geese were absent and, using the photo ID, we identified consecutive photos or single photos on which geese were absent. From this we obtained on the number of absences per nest per day and the summed length of these absences. Furthermore, we scored female preening (female is preening her feathers, bill in feathers) and when the female was busy with her nest (bill in nest material, often also her entire head is in the nest). Here, we will use the term nest maintenance to describe the latter behaviour in the remainder of the article. When a female has her head in the nest it might be that she is turning her eggs, but in passerine species an active search with the head dug into the nest material has been described and linked to nest sanitation against ectoparasites (Christe et al. 1996a). See Figure 2 for examples of photos that were taken.

During the experimental treatment, the geese stayed around the nest location and all birds returned after the nest was restored. Based on the wildlife camera pictures (see below, n = 20) the average time for females to resume incubation

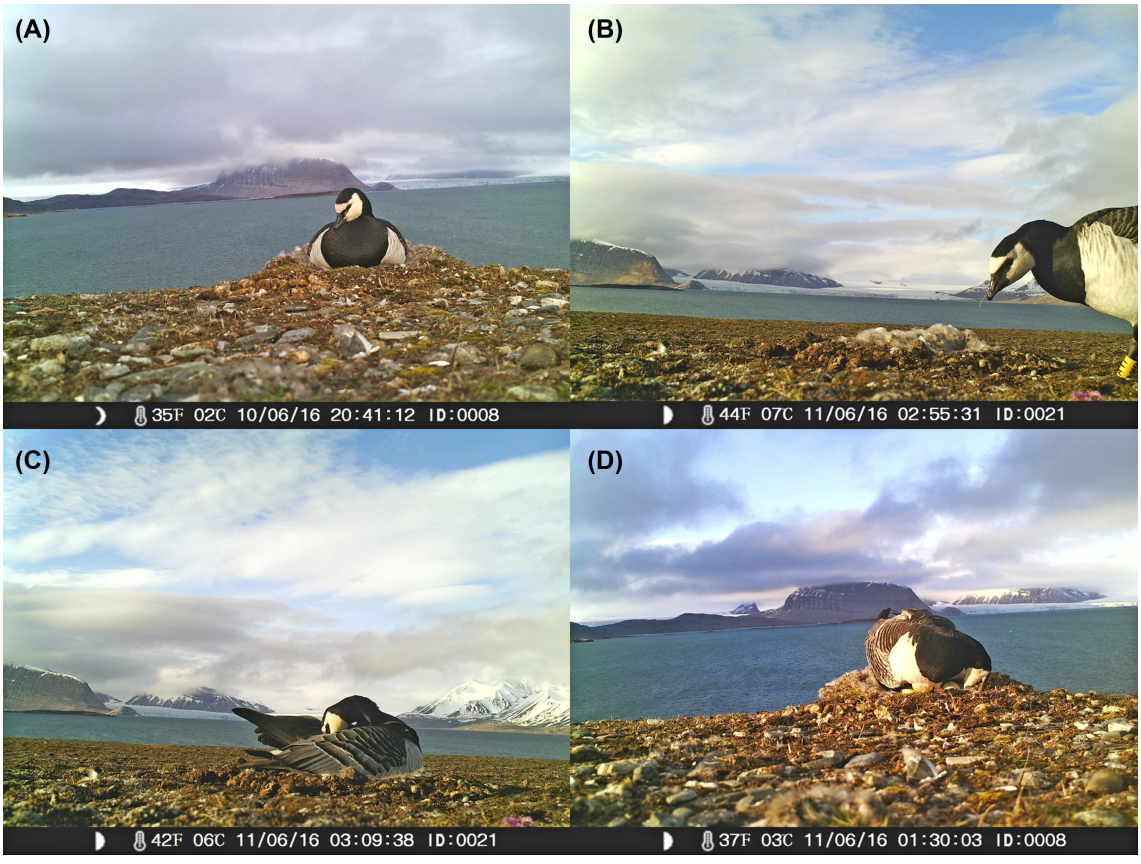

Figure 2. Examples of photographs from the wildlife cameras used for monitoring female behaviour showing (A) presence of the female on the nest, (B) absence of the female, (C) preening (female present) and (D) nest maintenance (female present). See Methods and Table 1 for an explanation of definitions.

after initial disturbance was 31 min, the minimum amount of time was 9 min and the maximum 1 h and 24 min.

The use of these data is based on some assumptions. We assumed that the cameras were able to detect when geese were leaving the nest and arriving back at the nest, and that the detection probability did not differ between the experimental groups. However, as the minimum interval of the camera to take a photo was set at 5 min, this might have hindered the accurate estimation of females leaving and arriving back at the nest (e.g. females might have moved in front of the camera, sat on the nest within 5 min and sat very still for a long time, escaping accurate detection of when she was present again on her nest). Therefore, we expected that the calculated time when females were absent, would be an overestimation. We compared our measurement with measurements of daily recess time (calculated from the average recess length and the average number of recesses per female) of Arctic barnacle geese performed by two studies (Eichhorn and Karagicheva 2008, Tombre et al. 2012). Based on our data, we calculated that the time a goose was absent was on average 157.1 min (SE = 15.9) per nest per day. Tombre et al. (2012) studied incubation behaviour in the same population and observed, during four 24-h cycles of 18 nests, an average recess length of 19.8 (SE = 1.2) min and a daily average number of recesses of 4.9 (SE = 0.5), which would on average give a daily recess time of 97.02 min. Eichhorn and Karagicheva (2008) observed 42 barnacle goose nests in Arctic Russia during bouts of 6–48 h in all periods of the day and incubation stages and found a daily recess time of 157 min. Thus, our measurement falls within the measurement of Eichhorn and Karagicheva (2008) but not within the measurement of Tombre et al. (2012). This is possible due to differences in methodology, measurement period and/or year differences. Overall, we judge that we can use our data to make a comparison between our experimental groups.

Statistics

All analyses were done in R ver. 3.4.2. We present model intercept ± standard error (SE), estimate (β) ± SE, F- or χ^2-test statistics with degrees of freedom (df) and group means and standard deviations (SD) in text or table where appropriate.

Blood coverage and nest parameters

We examined the association between blood coverage and two breeding parameters, clutch size and hatching success, using generalized linear mixed effects models (GLMMs) with a Poisson error distribution for the first and a binomial error distribution for the latter (glmer function in the R package lme4: Bates et al. 2015). We investigated the association between egg blood coverage and the number of hatchlings seen using GLMMs, with the number of eggs not seen as hatchlings (failures) and the number of hatchlings (successes) combined in a two-vector response variable, and a binomial error structure (Crawley 2007). We used a similar model for the analysis of the association between egg blood coverage and partial egg predation, with the number of eggs predated (failures) and the number of eggs not predated (successes) combined in a two-vector response variable. We added blood coverage as a continuous predictor in these models as we were interested in the direction of the effect. We added island and year as random effects to account for possible differences between the two islands and between the years. Binomial error bars for the graph on hatching success were calculated using the binom.confint function with the Wilson method in R (Dorai-Raj 2014).

Experiment to reduce flea numbers

We investigated the effect of microwaving nests on adult flea and flea larvae numbers using GLMMs with a Poisson error distribution. The logarithm of the dry mass of the nest sample was included as an offset in the models. Including a logarithm of exposure in this model (here dry mass of the nest sample) was useful as we were interested in the rate of flea and flea larvae numbers per dry nest mass. An offset is similar to including a regression predictor, but its coefficient is fixed to the value 1 (Crawley 2007, Gelman and Hill 2007). Furthermore, the interaction between treatment and the moment when the sample was taken (before, after (2 d) and longer after (24 d) the experiment) were added as factors. As a random effect we added nest identity, as nests were repeatedly measured. We checked for overdispersion, and when this was the case we added an individual-level random effect to the model to account for this (Agresti 2002). We made post-hoc comparisons by computing estimated marginal means (predicted marginal means) for the final models using the R package emmeans (Lenth 2018). To illustrate the experimental effects on the numbers of adult fleas and flea larvae per nest material sample graphically, we calculated the numbers per 100 g nest material to give a more straightforward measure.

Experimental effects on nest parameters

Hatching success data showed clear separation as all control nests hatched, but 3 flea-reduced nests did not (also referred to as the Hauck–Donner effect: Hauck and Donner 1977). Therefore, to investigate whether hatching success was significantly different between the experimental groups, we examined the effects of the experiment on hatching success using the Fisher's exact test. We investigated the experimental effect on the number of hatchlings seen using generalized linear models (GLMs) with a binomial error structure. We therefore combined the number of eggs not seen as hatchlings (failures) and the number of hatchlings seen (successes) in a two-vector response variable. We used a similar model to investigate the experimental effect on partial egg predation using by combining the number eggs predated (failures) and the number of eggs not predated (successes) in a two-vector response variable. We added the factor treatment as a predictor in these models. In case of overdispersion we fitted a quasibinomial error structure (Crawley 2007).

Experimental effects on female behaviour

First, to investigate whether the experimental groups differed in the total number of movements made by the geese as an indication for irritation, we analysed whether there was a difference in the total number of photos taken of control and flea-reduced nests per day (irrespective of photo quality). Therefore, we used a GLMM with a Poisson error distribution and we analysed the number of photos taken when the geese were present on their nest (Table 1 for an overview of the measurements). Second, we wanted to find out whether the experimental groups differed in the number of absences per day and the absence time per day. We analysed this data with a GLMM with a Poisson error distribution for the first and a linear mixed-effects model (LMM: lmer function in the R package lme4; Bates et al. 2015) for the latter. We used log-transformed absence time to meet model assumptions. Third, to investigate whether there was a difference between the experimental groups in anti-parasite related behaviours we investigated the probability that the detected behaviour was preening or nest maintenance. We analysed this data using GLMMs with a binomial error distribution. For the analyses of the number of photos, preening and nest maintenance, we selected photos when the female was present on her nest as this was when all geese were visible (some geese might have stood up preening in front of the camera, but other geese might have done so out of sight of the camera). In all these models, we added an interaction between the factor treatment and the time in days since the experiment as fixed effects. We added a random slope to the model when the random slope model was significantly better than a model with only a random intercept model. This was the case for almost all models. The random part in these allowed individuals to differ in the intercept as well as the slope of their reaction over time, thereby preventing overconfident estimates (Schielzeth and Forstmeier 2009). We checked for overdispersion, and when this was the case we added an individual-level random effect to the model to account for this (Agresti 2002). For all measures we checked whether there were differences between the groups before the experiment (−1 and −2 d before) and, in separate models, we investigated whether there were experimental effects by using the data with day −1 as a starting point.

Effects on nest temperature

To investigate the effects of treatment on daily average nest temperature and nest temperature fluctuations, we fitted linear mixed effects models. We added the interaction between the factors treatment and measurement day as fixed effects and nest identity as a random effect.

Data deposition

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.80n9608> (de Jong and Loonen 2019).

Results

Observational data on flea abundance and nest success

Correlations between egg blood coverage and nest parameters

We did not detect any relationship between egg blood coverage and clutch size (Table 2). Of 846 nests of which blood coverage was determined from 2012 to 2016, 86 nests had lost one or more eggs during incubation. There was also no correlation between egg blood coverage and partial egg predation. However, with increasing blood coverage on eggs, hatching success decreased significantly from 95% of nests hatched with no blood coverage to 83% of nests hatched with eggs fully covered in blood (Fig. 3, Table 2). We did not detect any correlation between egg blood coverage and the number of hatchlings in the nest (Table 2). We encountered on average three hatchlings per nest in 380 nests in total.

Experimental data

Experimental effects on nest parameters

We detected partial predation during incubation for six nests of both groups. The number of eggs predated versus the number of eggs not predated did not differ between the groups (average % of eggs predated (95% binomial confidence interval); control nests: 7.5% (4.1–13.2), flea-reduced nests: 8.9% (5.0–15.2). Intercept = 2.518 ± 0.478, β flea-reduced = -0.188 ± 0.662, $F_{1,57} = -0.171$, $p = 0.776$). Flea-reduced nests hatched on average 12.4 d (SD = 2.9) and control nests 11.4 d (SD = 2.7) after the experiment, which

Table 1. Overview of measurements taken from the wildlife camera data, their definitions, the data type and what models were used to analyse the data.

Measurement	Definition	Type and model
Number of photos	All photos that were taken during the experiment when geese were present on their nest, on days when they were not disturbed by researchers and when this day was at least one day before hatching.	Poisson: GLMM
Number of absences	The summed number of absences per nest per day extracted from single or consecutive photos when geese were absent.	Poisson: GLMM
Time absent	The summed time the female was absent in seconds per day extracted from time between consecutive photos when geese were absent.	Log-transformed: LMM
Preening	Female is on her nest preening her feathers (bill in feathers). Absence or presence of this behaviour.	Binomial: GLMM
Nest maintenance	Female is on her nest with her bill in the nest material, often also her entire head is in the nest. Absence or presence of this behaviour.	Binomial: GLMM

Table 2. ANOVA table for GLMMs on the correlation between egg blood coverage and nest parameters. Variables in bold font were kept in the final model. Values for non-significant predictors represent values just before removal in backward elimination.

Measurement	Variable	β	SE	χ^2	df	p
Clutch size	Intercept	1.280	0.054	–	–	–
	Blood coverage	0.020	0.018	1.253	1, 4	0.263
Partial egg predation	Intercept	3.689	0.472	–	–	–
	Blood coverage	−0.032	0.102	0.099	1, 4	0.753
Hatching success	Intercept	3.189	0.246	–	–	
	Blood coverage	**−0.341**	**0.131**	**6.540**	**1, 4**	**0.011**
Number of hatchlings	Intercept	3.689	0.472	–	–	–
	Blood coverage	−0.033	0.102	1.593	1, 4	0.207

was not significantly different (intercept = 11.367 ± 0.510, β flea-reduced nests = 1.073 ± 0.756, $F_{1,54} = 2.015$, p = 0.162). We found no significant difference between control and flea-reduced nests in hatching success, although flea-reduced nests hatched slightly less well than control nests (control: 30/30 hatched, flea-reduced: 25/28 hatched; p = 0.106). In 19 control and 21 flea-reduced nests, we encountered hatchlings. The number of hatchlings seen in these nests was not different between the groups (average % of eggs seen as hatchlings (95% binomial confidence interval): control nests = 50.7% (42.4–59.1), flea-reduced nests = 62.9% (54.1–70.9). Intercept = 1.386 ± 0.332, β flea-reduced nests = 0.331 ± 0.486, $F_{1,39} = 0.468$, p = 0.494).

Experimental effects on female behaviour

For all measurements, we did not detect a difference between the experimental groups before the experiment was started. The number of photos taken when the goose was present decreased for flea-reduced nests over time in comparison to control nests (Table 3, Fig. 4). However, the model containing this interaction was not significantly better than the null model ($\chi^2_{3,8} = 7.63$, p = 0.054), indicating that this effect was not very strong. A median number of 68 photos were taken of control nests and 59 of flea-reduced nests per day. Both females with control and flea-reduced nests were an equally number of times per day absent from the nest (Table 3; control: median = 5, flea-reduced: median = 6). The number of absences decreased over time irrespective of treatment. Control females and flea-reduced females did not differ in the time they were absent from the nest (back calculated from log; control: mean = 118.3 min d^{-1}, flea-reduced: mean = 119.9 min d^{-1}). When on the nest, control females were seen preening on average 14.8% of the photos (binomial 95% confidence interval: 13.8–15.8) while flea-reduced females preened less, as they were seen preening on average 12.0% of the photos (binomial 95% confidence interval: 11.0–13.0). However, we did not detect this difference in the analysis of the data over time (Table 3). Detection of nest maintenance did not differ between the groups (Table 3; control: 10.8% of the photos (binomial 95% confidence interval: 10.0–11.7), flea-reduced: 10.7% of the photos (binomial 95% confidence interval: 9.7–11.7), but decreased significantly over time irrespective of treatment.

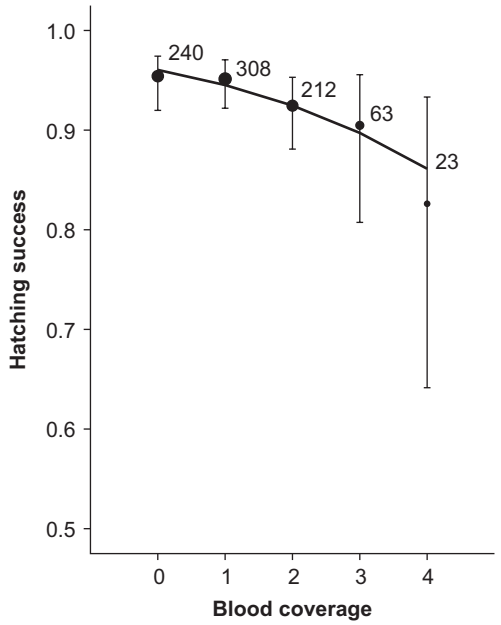

Figure 3. The correlation between egg blood coverage (from 0 no blood, to 4 fully covered in blood) and hatching success of Arctic breeding barnacle geese. Sample size is indicated by symbol size and the numbers next to the error bars. Error bars depict 95% binomial confidence intervals.

Experimental effects on nest temperature

We did not detect differences between the control and flea-reduced group in average nest temperature ($\chi^2_{1,6} = 0.047$, p = 0.828) or nest temperature fluctuations ($\chi^2_{1,6} = 0.435$, p = 0.510) before or after the experiment (Table 4). Daily average nest temperature did increase over time ($\chi^2_{2,5} = 29.851$, p < 0.001).

Discussion

In this study we investigated the effects of nest fleas on the behaviour and reproductive success of a precocial species; Arctic breeding barnacle geese. First, we examined whether there was an association between flea abundance (estimated by egg blood coverage) and several parameters of reproductive success. Second, we experimentally reduced nest fleas in a subset of nests by using a heat-treatment and

Table 3. ANOVA table for the GLMMs on the total number of photos, the total number of absences, female preening and nest maintenance and for the LMM on the total time absent for barnacle goose females with control and flea-reduced nests. Values for non-significant predictors represent values just before removal in backward elimination. Intercepts are given from minimal adequate model. Variables in bold stayed in the final model.

Measurement	Variable	β	SE	$\Delta\chi^2$	df	p
Number of photos	Intercept	4.066	0.201	–	–	–
	Treatment			0.151	1, 7	0.697
	Flea-reduced	0.217	0.285			
	Time since experiment	0.008	0.033	2.801	1, 6	0.094
	Treatment flea-reduced × Time since experiment	**−0.108**	**0.048**	**7.63**	**1, 8**	**0.031**
Number of absences	Intercept	1.820	0.087	–	–	–
	Treatment			0.096	1, 4	0.757
	Flea-reduced	−0.049	0.156			
	Time since experiment	**−0.035**	**0.011**	**10.168**	**1, 3**	**0.001**
	Treatment flea-reduced × Time since experiment	−0.013	0.022	0.320	1, 5	0.572
Time absent	Intercept	8.889	0.095	–	–	–
	Treatment			0.007	1, 7	0.935
	Flea-reduced	−0.018	0.195			
	Time since experiment	−0.038	0.026	2.136	1, 6	0.144
	Treatment flea-reduced × Time since experiment	0.045	0.053	0.809	1, 8	0.369
Female preening	Intercept	−1.966	0.142	–	–	–
	Treatment			0.507	1, 6	0.477
	Flea-reduced	−0.196	0.274			
	Time since experiment	−0.026	0.027	0.857	1, 5	0.355
	Treatment flea-reduced × Time since experiment	−0.078	0.051	2.191	1, 7	0.139
Nest maintenance	Intercept	−2.16	0.218	–	–	–
	Treatment			0.828	1, 6	0.363
	Flea-reduced	−0.281	0.307			
	Time since experiment	**−0.154**	**0.041**	**11.531**	**1, 5**	**0.001**
	Treatment flea-reduced × Time since experiment	−0.028	0.08	0.125	1, 7	0.724

compared anti-parasite and incubation behaviours of female geese with control and flea-reduced nests. We expected that female geese with natural levels of flea-infestation in their nests would show increased anti-parasite behaviours, an increased frequency and/or length of incubation breaks and a lower nest success relative to the females with flea-reduced nests. In line with previous work on Ross's and lesser snow geese (Harriman and Alisauskas 2010), we detected a negative association between egg blood coverage, used as a proxy for the level of flea infestation, and hatching success in the Spitsbergen barnacle geese. We found little support, however, for changes in incubation behaviour as a possible mechanism to explain this effect. The number of photos taken by the wildlife camera upon movement, decreased over time for females with flea-reduced nests, while the number of photos stayed similar for females with control, flea-infested, nests. Females incubating on flea-reduced nests thus seemed to move relatively less while on the nest. This difference was however not significant. Also, females with flea-reduced nests seemed to preen slightly less, but this was not apparent in the analyses of the overall dataset. Females with control or flea-reduced nests further did not differ in the number of absences, their time absent, the proportion of photos on which nest maintenance was observed or their nest temperature. Thus, though some suggestions were found for differences in female behaviour as a consequence of flea infestation, we found no clear effects.

One possibility is that there were effects of fleas on female anti-parasite and incubation behaviour, but we were unable to detect these as the effect size was too small given our current sample size. We have some indications for small effects of fleas on the number of movements and preening and if our sample size would have been larger we may have been able to better estimate whether these small effects can be significant (Forstmeier et al. 2017). Below, we discuss other options for why we found no clear evidence to support our hypotheses.

Is it likely that fleas can exert effects?

While predators kill their prey and consume them completely, parasites, though often ubiquitous, may not always have obvious effects on their host (Newton 1998). Bird nests are an ideal environment for a range of ectoparasites such as ticks, mites, lice, flies, bugs and fleas, as they have a relatively stable micro-environment and provide a predicable food source. This predictable food source first consists of the incubating bird, that is exposed to the nest-dwelling parasites through close contact with the nest material during the lengthy incubation period (López-Rull and Macías Garcia 2015). As most avian species also develop a bare, highly vascularized, brood patch on the ventral abdominal area to aid in heat-transfer to the eggs (Lea and Klandorf 2002), they provide an 'easy target' for ectoparasites (López-Rull and Macías Garcia 2015). After hatching, nestlings are available

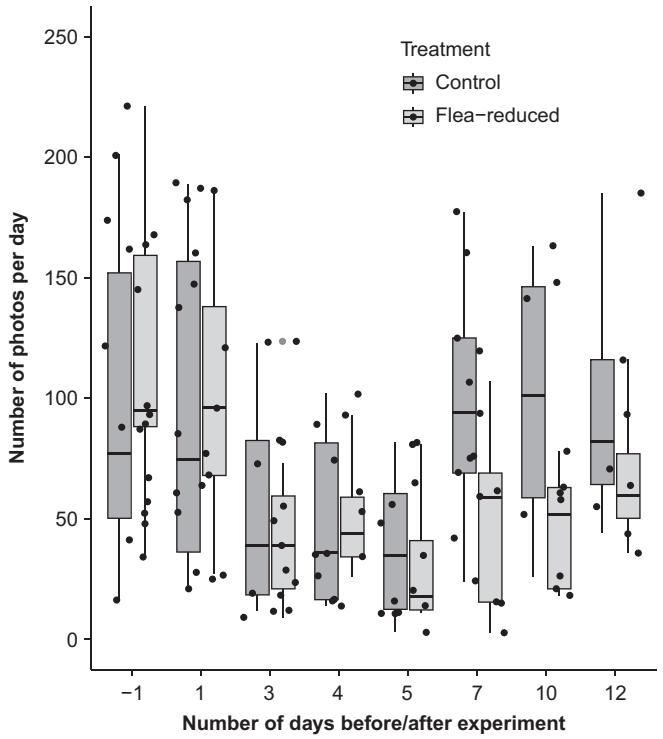

Figure 4. Difference between the number of photos taken of females with control and flea-reduced nests over time. Points indicate values of individual nests. Boxes show medians as well as 25% and 75% quartiles. Whiskers indicate the range between the 10th and 90th percentiles.

as a food source for the nest parasites (López-Rull and Macías Garcia 2015). Especially nestlings of altricial birds are relatively helpless as they are not very mobile, cannot leave the nest to escape parasites until they fledge, are not able to remove parasites from their body and their physiological defences are not yet entirely developed (Starck and Ricklefs 1998, Adelman et al. 2013). Several studies have detected quite substantial negative effects of ectoparasite abundance on fitness measures in altricial bird species (see López-Rull and Macías Garcia, 2015 and references below). Parents may be directly affected by ectoparasites when attending to the brood through possibly e.g. body mass loss, tissue/blood loss, feather loss, infection with pathogens and an increase in time spent on parasite removal, resulting in a decrease in survival (Brown et al. 1995). Furthermore, also, indirect effects on parental fitness components can play an important role. For instance, several studies have found that young beg more when they are in infested nests, parents in response increase their food supply accordingly (Christe et al. 1996b, Tripet and Richner 1997, Hurtrez-Boussès et al. 1998) and, as a consequence, can have a reduced future reproductive success (Richner and Tripet 1999). Furthermore, experimentally increased nest ectoparasite abundance has been shown to decrease e.g. nestling growth and survival (Richner et al. 1993, Møller 1994, Cantarero et al. 2013). In precocial birds, it is to be expected that direct effects on offspring are limited, but that there could be substantial negative effects of ectoparasites on attending parents (Harriman and Alisauskas 2010). Interestingly, López-Rull and Macías Garcia (2015) propose that susceptibility to nest-dwelling ectoparasites may be a major driver promoting and/or maintaining precociality. As far as we know, only three previous studies have investigated effects of ectoparasites on reproductive success in precocial bird species. Hoodless et al. (2003) and Baines and Taylor (2016) detected positive effects of treatments against ticks on clutch survival in pheasants *Phasianus colchicus* and chick survival in red grouse *Lagopus lagopus scotica* respectively. Harriman and Alisauskas (2010) detected a negative association between nest fleas and reproductive success in a population of Ross's and lesser snow geese in Arctic Canada. Similarly, in our study in a Spitsbergen barnacle goose population we also detected such an association. The above discussed scientific evidence thus indicates that ectoparasite presence on precocial birds can be associated with negative reproductive success. It must be noted however that experimental work investigating the effects of fleas in precocial birds has up to now not been done. In our experiment we did not find any effects of nest fleas on female behaviour or hatching success. What can explain this apparent discrepancy between the observational data, showing a negative relationship between fleas and hatching success, and our experiment in which we did not find any effects? Below we discuss several possibilities.

Did our experiment work?

Experiments have often been used to separate possible effects of the individual, and the resources it is able to obtain, on individual fitness (Both and Visser 2000). We adapted a method previously used to effectively eliminate nest parasites from nests of nest-hole breeders, namely heat-treatment of nests (Richner et al. 1993). Using this method, we were able to successfully decrease the adult flea numbers that take

Table 4. Daily average nest temperature (average T in degrees Celsius) and fluctuations in nest temperature (average T fluctuation) measured on two days before and three and nine days after the experiment in control and flea-reduced nests. Sample size is indicated between brackets.

	Control		Flea-reduced	
Days before/after experiment	Average T ± SD (n)	Average T fluctuation ± SD	Average T ± SD (n)	Average T fluctuation ± SD
−2	26.3 ± 3.6 (29)	1.2 ± 0.8	26.5 ± 3.2 (26)	1.0 ± 0.4
3	27.8 ± 3.7 (26)	1.4 ± 1.3	27.9 ± 3.6 (23)	1.4 ± 0.8
9	30.2 ± 2.1 (17)	1.2 ± 0.3	30.6 ± 2.3 (9)	1.2 ± 0.3

blood from the incubating bird. When measured 2 d after the experiment heat-treated nests had significantly less fleas and flea larvae than control nests and this difference still there after 24 d. Yet, as larvae stay deep in the nest and do not take blood themselves (Rothschild and Clay 1952) they are likely not directly irritating for the incubating birds. Despite that the experimental treatment was successful in decreasing nest flea numbers, we detected no large effects on female behaviour or nest parameters. A possible reason for this lack of effects is that the experiment started relatively late; nests hatched approximately 12 d after the heat-treatment, while the entire period of nest attendance in barnacle geese from the first egg is approximately 29 d. This late removal of fleas might have been unable to affect nest parameters such as hatching success, that could already have been influenced by flea infestation. Overall, hatching success of experimental nests was actually very high. While effects on hatching success may have not been apparent due to the short period the experiment lasted, we expect that this would not have been a problem for detecting possible effects on female behaviour. Flea numbers were nearly absent in flea-reduced nests and abundant in control nests. We expect that females would have been able to notice this (Hawlena et al. 2008) and if indeed female behaviour is affected by nests fleas, we expect we would have picked up on this. Repeating the experiment would provide a stronger basis to the knowledge on effects of ectoparasites on precocial birds.

Are alternative mechanisms at play?

So if our experiment worked, why then did we detect a relationship between flea abundance and hatching success in our breeding colony, but have no experimental evidence for effects on female breeding behaviour underlying this effect? It is possible that some other mechanism has caused the observed negative association between egg blood coverage and hatching success. One other proposed mechanism is that egg pores are blocked by dried-up blood in heavily flea-infested nests and thereby reduce effective gas exchange and subsequently lower embryo survival (Harriman and Alisauskas 2010). Reduced gas exchange, induced by partially wrapping eggs with an impermeable film after 14–18 d of incubation, negatively affected hatch rate and caused cognitive deficits in chickens (Rodricks et al. 2004). In our study area we have no data to judge whether effects of blood coverage on the gas-exchange of eggs were at play, future work should focus on testing this hypothesis. Another possibility is that nests with blood-covered eggs are more likely to attract predators due to the scent of blood (Harriman and Alisauskas 2010). In our study area foxes have not been able to enter the breeding islands since 1995 due to early breakup or non-existence of a sea-ice connection to the mainland (Hübner et al. 2002). However, the number of polar bears *Ursus maritimus* that have been sighted in Kongsfjorden on the breeding islands have increased and, when they are present, often cause the destruction of complete clutches (Prop et al. 2015). It could be a possibility that polar bears are attracted to blood scent. Other egg predators that are present on the island are glaucous gulls *Larus hyperboreus*, Arctic skua *Stercorarius parasiticus* and great skua *Stercorarius skua* (Hübner et al. 2002). These aerial predators can rapidly take eggs when a nest is left unattended (Prop et al. 1984). As it is difficult to separate nest desertion from nest predation, we investigated partial egg predation during incubation. We did not find an association between egg blood coverage and partial egg predation, nor were there differences in partial egg predation between the experimental groups. This indicates that at least partial egg predation, which most likely is caused by aerial predators, is not affected by egg blood coverage.

Can quality differences between parents play a role?

Next to fleas exerting negative effects on goose hatching success via the above mentioned alternative mechanisms, it could also be that other factors associated with flea infestation are the actual cause of the effect (third variable problem). One possibility is that flea infestation is correlated with parental quality. It could be that lower quality parents have more nest fleas and the negative effect of nest fleas on hatching success is actually an effect of parental quality rather than directly of the fleas. Harriman and Alisauskas (2010) also discussed the possibility that hosts differ in quality in the light of their finding of a negative association between high flea abundance and reproductive success in two goose species. They argued that there were no indications in their study that fleas aggregate on low quality hosts, as fleas were more abundant in areas of the colony with a longer history of nesting and in nests with more eggs and there was no correlation between female mass and egg blood coverage (Harriman et al. 2008, Harriman and Alisauskas 2010). We did not detect a significant correlation between egg blood coverage and the number of eggs in the nest in our study, but there was a similar trend that nests with bloodier eggs contained more eggs. Geese that arrive in good condition at the breeding grounds can start nesting earlier and lay larger clutches (Bêty et al. 2003). Therefore, if even, higher quality birds might acquire more nest fleas when they return to certain good areas in the colony with a longer history of nesting. Another option is that fleas are doing better when feeding on high quality birds (Dawson and Bortolotti 1997, Christe et al. 2003). Thus, in our colony, if anything, flea abundance may have a positive association with parental quality, which would not serve as an explanation for the negative effect of blood coverage on hatching success. Specific experiments in which both the effect of parental quality and the effect of nest fleas are independently estimated are needed to solve this conundrum.

Conclusion

Our findings provide new important evidence that nest fleas and reproductive success of a precocial species are negatively associated. The mechanism, however, remains unknown. The outcome of our experiment indicated that negative effects of nest fleas on hatching success are likely not mediated through

effects of nest fleas on female incubation behaviour. Perhaps other mechanisms such as a decrease in gas exchange or total clutch predation play a role. However, it is also possible that a third variable, such as quality differences between goose parents, may be an important mediator. Further experimental work is needed on precocial species to understand the mechanism behind the negative effect of nest fleas on hatching success. Knowledge of the exact mechanism is vital to understand how parasites can affect individual fitness. Especially, in a changing Arctic, parasite abundance may increase and become a bigger force to reckon with in understanding species population dynamics.

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