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In kittiwakes food availability partially explains the seasonal decline in humoral immunocompetence

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Summary

1. The immune system plays an important role in fitness, and interindividual variation in immunocompetence is due to several factors including food supply.
2. Seasonal variation in food resources may therefore explain why immunocompetence in bird nestlings usually declines throughout the breeding season, with chicks born early in the season receiving more food than chicks born later, and thereby possibly developing a more potent immune system. Although there are studies supporting this hypothesis, none has been experimental.
3. We performed an experiment in the kittiwake *Rissa tridactyla* by manipulating the food supply of pairs that were left to produce a first brood, and of pairs that were induced to produce a late replacement brood.
4. If food supply mediates, at least partially, seasonal variations in chick immunocompetence, non-food-supplemented chicks would show a stronger seasonal decline in immunocompetence than food-supplemented chicks.
5. Food supplementation improved humoral immunocompetence (the production of immunoglobulins Y), but not T-cell immunocompetence (phytohaemagglutinin, PHA response). T-cell immunocompetence of food-supplemented and non-food-supplemented chicks decreased through the season but to a similar extent, whereas the humoral immunocompetence of non-food-supplemented chicks decreased more strongly than that of food-supplemented chicks.
6. Our results suggest that the seasonal decline in humoral immunocompetence can be explained, at least partly, by variations in food supply throughout the breeding season.

Key-words: food availability, hatching date, immune system, *Rissa tridactyla*, seasonal variation

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Introduction

The vertebrate immune system has evolved as a defence mechanism against invading pathogenic microorganisms (Goldsby, Kindt & Osborne 2000) and hence plays a critical role in host survival and reproductive success (Frank 2002). Not all individuals are affected to the same degree by the negative impact of parasites because they are typically not equally able to resist infestation by parasites because of interindividual variation in genetics (e.g. Brinkhof *et al*. 1999; Roulin *et al*. 2000), non-genetic maternal effects (Gasparini *et al*. 2001, 2002; Grindstaff, Brodie III & Ketterson 2003) and other factors affecting the general phenotypic condition. Among the latter factors, food availability can profoundly alter the quality of the immune system because mounting an immune response is costly (Bonneaud *et al*. 2003) and requires nutrients and energy resources, particularly in young individuals whose physiological functions are maturing at the same time (Klasing 1998; Alonso-Alvarez & Tella 2001). Therefore, spatial and temporal variation in the availability of food resources may cause seasonal changes in the quality of the immune response to parasites.

Studying the relationship between food availability and the immune system is difficult in natural populations (Alonso-Alvarez & Tella 2001). Two experimental studies have investigated the effect of breeding date on the immune system by inducing European Magpies, *Pica pica*, to produce a replacement clutch after the first clutch was artificially removed (Sorci, Soler &

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Møller 1997; De Neve *et al*. 2004). The finding that an index of T-cell immunocompetence was lower in nestlings from replacement broods compared with nestlings from first broods was interpreted as a direct effect of a decrease in the availability of food resources at the end of the breeding season (but see Merino *et al*. 2000; Jovani *et al*. 2005). While that interpretation may be correct, these experiments do not rule out the possibility that parents invested less effort in producing replacement eggs and offspring compared with their first breeding attempt, which in turn reduced immunocompetence in replacement offspring. Life-history theory predicts that reproductive investment should decrease throughout the season because the residual reproductive value of offspring commonly decreases with date (Stearns 1992). Furthermore, parents should allocate a large proportion of resources to the first breeding attempt as the likelihood of producing a replacement brood is commonly low (Hipfner, Gaston & Storey 2001; but see De Neve *et al*. 2004). The experimental design developed by Sorci *et al*. (1997) and De Neve *et al*. (2004) would therefore be strengthened by manipulating food resources, e.g. by experimentally providing food to half the pairs from first and replacement breeding attempts. If seasonal variation in immunocompetence is due to seasonal variation in food supply, the reduction in nestling immunocompetence throughout the season should be stronger in the non-food-supplemented chicks than in food-supplemented ones.

In the present study, we performed such an experiment with the Black-Legged Kittiwake (*Rissa tridactyla*), a colonial seabird species that is subject to pronounced natural variations in food availability (Oro & Furness 2002), which may account for variation in its capacity to mount an immune response (McCoy *et al*. 2002). We randomly allocated half the breeding pairs into a 'first brood' group that was allowed to complete the first breeding attempt, and the other half in a 'replacement brood' group for which we artificially removed the first clutch to induce production of a replacement clutch. This design therefore generates groups of birds that breed early *vs* late in the season. In each of these two groups, we allocated half the breeding pairs to each of two treatments: food supplementation *vs* non-food supplementation. As our aim was to investigate whether a decline in food availability throughout the breeding season causes a reduction in immunocompetence, we food-supplemented breeding pairs throughout a large portion of the breeding season; prior to egg-laying and through chick-rearing (to 21 days of age). To assess whether seasonal variation in food supply affects different components of the immune system, we measured T-cell immunocompetence with the phytohaemagglutinin (PHA) assay in 20-day-old chicks as in Sorci *et al*. (1997) and De Neve *et al*. (2004) and humoral immunocompetence by measuring total immunoglobulin in 10-day-old chicks. If the decrease in immunocompetence throughout the season is due to a decrease in food resources, as advocated by Sorci *et al*. (1997), we predicted that the seasonal decline in both immune components should be more pronounced in the non-food-supplemented chicks than in the food-supplemented chicks; the food supplementation could indeed compensate for any seasonal difference in the access to food resources. This prediction was based on the assumption that the food supplementation experiment enhances immunocompetence.

Materials and methods

STUDY SITE

The study was conducted at a kittiwake colony on Middleton Island (59°26′N, 146°20′W) in the northern Gulf of Alaska (Gill *et al*. 2004). About 12 000 pairs of kittiwakes breed on cliffs and on several artificial structures such as an abandoned US Air Force radar tower that has been modified to provide highly accessible nest sites for use in experiments (Gill & Hatch 2002). Nests can be viewed and accessed from the inside of the tower through sliding panes of one-way mirror glass.

EXPERIMENTAL DESIGN

In the spring of 2002, we randomly allocated nests into two experimental treatments, food-supplemented ($N = 28$) and non-food-supplemented nests ($N = 29$). As in a previous study using a comparable design (Lanctot *et al*. 2003), adult males and females from the food-supplemented treatment were provided Capelin, *Mallotus villosus*, three times daily (08.00, 14.00 and 18.00 h) until satiation was reached. The mean number of fish ingested by the occupants of each nest could be recorded precisely as fish were provided one at a time. Supplemental feeding with capelin (average individual mass = 25 g), a high-quality natural prey of kittiwakes, began on 16 May and ended when chicks reached 21 days of age. We started the food supplementation treatment prior to egg-laying because food can improve offspring immunocompetence via nutrients put into the eggs, as well as via the transfer of food from parents to nestlings. Our aim was to determine whether differences in food resources over the entire breeding season could explain variation in nestling immunocompetence independently of whether this effect is due to prehatching maternal effects or to rearing conditions. On average, we provided Capelin to pairs $11{\cdot}0 \pm 0{\cdot}7$ days before they laid the first egg of their first clutch. We induced the production of a replacement clutch in 12 food-supplemented pairs (all of them produced a replacement clutch) and in 13 non-food-supplemented pairs (11 of them produced a replacement clutch) by removing eggs $6{\cdot}7 \pm 0{\cdot}5$ days (range: 5–14) after the first clutch was complete. The number of days was not different between the two food treatments (Student's t-test, $t_{23} = 0{\cdot}93$, $P = 0{\cdot}36$). Although the average number of supplemented fish consumed per day was similar between pairs that produced only a first brood

and pairs that produced an additional replacement brood (2·2 ± 0·3 *vs* 2·8 ± 0·3 fish day^{-1}; *t*-test, $t_{20} = 1{\cdot}41$, $P = 0{\cdot}17$), the total number of supplemented fish consumed over the experiment was higher for pairs that produced an additional replacement brood (215 ± 24 *vs* 137 ± 21 fish, $t_{20} = 2{\cdot}39$, $P = 0{\cdot}03$; range: 104–345 *vs* 36–278).

Kittiwake clutches contain one to three eggs and, within a clutch, the first laid egg was denoted A, the second B and third C. Because clutch size differs between the first and replacement breeding attempts, we removed the B and C eggs so that each brood contained a single chick that hatched from an A egg. In this way, first and replacement broods did not differ in the level of sibling competition, hatching asynchrony and prehatching maternal effects (maternal investment in the first, second and third laid eggs is known to differ; Nager, Monaghan & Houston 2000). Adult birds involved in the experiment were not all individually marked to avoid disturbance that could have interfered with our experiment (e.g. willingness to accept extra fish), but pairs that were induced to lay a replacement clutch were assumed to have done so in the same nest. Among the nine pairs where adults were individually marked (we could read their colour ring without capturing the birds), all of them were indeed recorded brooding a replacement clutch on the same nest. Furthermore, evidence of nest site fidelity between first and induced replacement clutch come from data gathered at a colony in northern Norway, where 83 marked birds out of 85 were recorded brooding a replacement clutch in the nest where they had laid their first clutch (J. Gasparini and T. Boulinier, unpublished data). This was also the case at a colony with marked kittwakes in England (see Wooller 1980). These results indicate that kittiwakes show a strong site fidelity between first and replacement clutches. Nests were monitored daily to determine laying and hatching date. As ensured by our experimental procedure, the hatching dates of replacement broods (mean: 9 July) were later than the ones of first broods (mean 25 June), but did not differ between feeding treatments (two-way ANOVA: first *vs* replacement broods: $F_{1,39} = 168{\cdot}10$, $P < 0{\cdot}0001$; food treatment: $F_{1,39} = 0{\cdot}01$, $P = 0{\cdot}92$; interaction: $F_{1,39} = 0{\cdot}22$, $P = 0{\cdot}64$).

HUMORAL IMMUNOCOMPETENCE

To obtain a measure of the humoral immune system, we measured the concentration of plasma immunoglobulins Y (IgY) in growing chicks. We preferred this approach to the measurement of a specific immune response elicited by an injection of a non-pathogenic antibody (e.g. Roulin *et al*. 2000) because plasma concentration in IgY reflects the quantity and overall efficiency of B-lymphocytes (Apanius 1998). In contrast, an immune response towards a specific antigen could reflect the ability to control the proliferation of a single pathogen out of a large suite of microorganisms and parasites found in the environment of any animal. In hatchling chickens, the concentration of maternal IgY transferred through the egg is high. Five days after hatching, B cells of chicks start to produce IgY and the concentration of endogenous IgY increases sharply to a plateau at 20 days old. At 10 days of age, the proportion of residual maternal IgY is negligible, and the production of endogenous IgY is to a large extent not induced by natural antigens (Apanius 1998). In fact, to induce the production of a detectable level of IgY by 10-day-old nestlings, an infection needs to occur before 3 days of age, a time when nestlings can barely mount a humoral immune response directed to a specific antigen (Apanius 1998). We therefore measured IgY blood concentration in 10-day-old kittiwake chicks. We took 0·1 ml blood from the ulnar vein using a sterile syringe rinsed with heparin. Blood plasma was immediately isolated by centrifugation and kept frozen until analysis in the autumn of 2002.

Total IgY concentration in blood plasma was determined using a sandwich ELISA (Crowther 2001). For the solid phase, we used a microtitre plate (8 × 12 well format, Microlon 600, Greiner, Frickenhausen, Germany). Each well was coated with 100 µl of a 2·3 µg ml^{-1} of rabbit antichicken IgG (Jackson Immunoresearch Laboratories, West Grove, PA, USA) diluted in phosphate-buffered saline (PBS) and incubated for 2 h at room temperature (RT). The plates were then washed three times with PBS. We saturated each plate with 200 µl PBS containing 1·5% bovine serum albumin (PBS-BSA) for 2 h at RT and washed again. 100 µl of the diluted plasma (1:1000) were distributed into the wells and incubated overnight at RT. After washing, 100 µl peroxydase-conjugated rabbit antichicken IgG (1:3000, Sigma, A-9046, St Louis, MO, USA, Martinez *et al*. 2003) in PBS-BSA were added and left for 2 h at RT. After washing five times, 100 µl peroxydase substrate (*o*-phenylenediamine dihydrochloride, 0·4 mg ml^{-1}, Sigma) were added for 15 min at RT and then stopped using 50 µl hydrochloric acid (HCl 1 M). Optical density (OD; our measure of IgY concentration) was read at 490 nm with a spectrophotometer. As a standard, a mixture of several kittiwake samples was measured in serial dilutions to correct OD values for a possible plate effect. After calibrations with the standard, there was a high repeatability between OD values of the same samples within ($r = 0{\cdot}85$, $F_{31,32} = 12{\cdot}01$, $P < 0{\cdot}0001$) and between plates ($r = 0{\cdot}92$, $F_{24,25} = 22{\cdot}76$, $P < 0{\cdot}0001$).

T-CELL IMMUNOCOMPETENCE

Since the immune system is more mature in 20-day-old chicks and variation in immunocompetence can be more easily assessed, we measured T-cell immunocompetence at that age (Apanius 1998). We obtained this measure by injecting 0·2 mg of PHA (Phytohaemagglutinin-P; Sigma, Lyon, France; mixed in 0·05 ml physiological saline 0·9% NaCl; Goto *et al*. 1978) in the centre of the right wing web (the skin in the joint between the carpometatarsus and the ulna/radius). The thickness

of the wing web was measured to the nearest 0·01 mm using a spessimeter (Teclock Co., Okaya City, Japan) before injection and exactly 24 h later. The difference in wing web thickness after and before injection was our index of T-cell immunocompetence of the chicks (Smits, Bortolotti & Tella 1999). To reduce measurement errors, wing webs were measured three times in a row and the average value was used in the statistical analyses. The repeatability of the three measurements was high ($r = 0{\cdot}97$, $F_{79,160} = 115{\cdot}48$, $P < 0{\cdot}0001$, $n = 80$ measurements done on 40 chicks).

STATISTICAL ANALYSES

Throughout the paper statistical tests were performed with SAS (1996). As we were interested in testing whether food supply accounted for a potential decrease in immunocompetence throughout the season, we entered hatching date as a covariate in ANCOVAs instead of using first *vs* replacement clutch as a factor (as done in Sorci *et al.* 1997). Variances in hatching date (the folded *F*-statistic, $F'_{19.22} = 1{\cdot}38$, $P = 0{\cdot}47$), T-cell ($F'_{19.22} = 1{\cdot}40$, $P = 0{\cdot}45$) and humoral immunocompetence ($F'_{19.22} = 1{\cdot}40$, $P = 0{\cdot}45$) were not different between food treatments, therefore we performed parametric statistical tests. Among the 43 chicks that successfully hatched, 25 were from first clutches (13 food-supplemented and 12 non-food-supplemented) and 18 from replacement clutches (10 food-supplemented and 8 non-food-supplemented). Three chicks from the non-food-supplemented treatment (one from a first clutch and two from replacement clutches) died during the course of the experiment, explaining differences in sample size between analyses. Significance levels were set to 0·05 and all tests were two-tailed. Means are given ± their standard error.

Results

HUMORAL IMMUNOCOMPETENCE

The seasonal decrease in humoral immunocompetence estimated by the IgY concentration at 10 days of age was not significantly different between food treatments (full ANCOVA model with the interaction between hatching date and food treatment: $F_{1,39} = 1{\cdot}54$, $P = 0{\cdot}22$; Fig. 1). Although the food treatment significantly increased the humoral immunocompetence (similar ANCOVA model without the interaction: $F_{1,40} = 5{\cdot}01$, $P = 0{\cdot}03$), it was not enough to offset the seasonal decline in this parameter (hatching date: $F_{1,40} = 6{\cdot}95$, $P = 0{\cdot}01$). It is possible the interaction was not significant because of a lack of statistical power, but the results suggest it is perhaps biologically significant; in non-food-supplemented chicks humoral immunocompetence significantly decreased through the season ($F_{1,18} = 10{\cdot}08$, $P = 0{\cdot}005$) while it did not in food-supplemented chicks ($F_{1,21} = 1{\cdot}41$, $P = 0{\cdot}25$). Results were similar when adding clutch sequence (first or replacement) in the model (full ANCOVA: interaction between food treatment and hatching date: $F_{1,37} = 1{\cdot}22$, $P = 0{\cdot}27$; similar ANCOVA after having removed the interaction: food treatment: $F_{1,37} = 4{\cdot}96$, $P = 0{\cdot}03$; hatching date: $F_{1,37} = 3{\cdot}51$, $P = 0{\cdot}07$; clutch sequence: $F_{1,37} = 0{\cdot}35$, $P = 0{\cdot}55$), suggesting that the apparent overall seasonal decrease in immunocompetence is better explained by an environmental factor such as food supply associated with season, rather than by a differential allocation of resources by parents between the two annual clutches. In similar analyses, where we added in turn clutch size, egg weight, total clutch weight or time needed to incubate eggs as covariates (all *P*-values > 0·37), the effect of hatching date on humoral immunocompetence remained significant ($P < 0{\cdot}02$). Although variances in hatching date between treatments were not significantly different (reported in the Methods section), Fig. 1 suggests some discrepancies. To assess whether this potential problem of heterogeneity of variances could have inflated the effect of hatching date on humoral immunocompetence, we checked *P*-values with a non-parametric ANCOVA (permutation test, see Manly 1997). In this test, we found the same patterns as when performing parametric analyses (interaction between food supplementation and hatching date: $P = 0{\cdot}21$; after removing the interaction: food supplementation: $P = 0{\cdot}02$, hatching date: $P = 0{\cdot}003$).

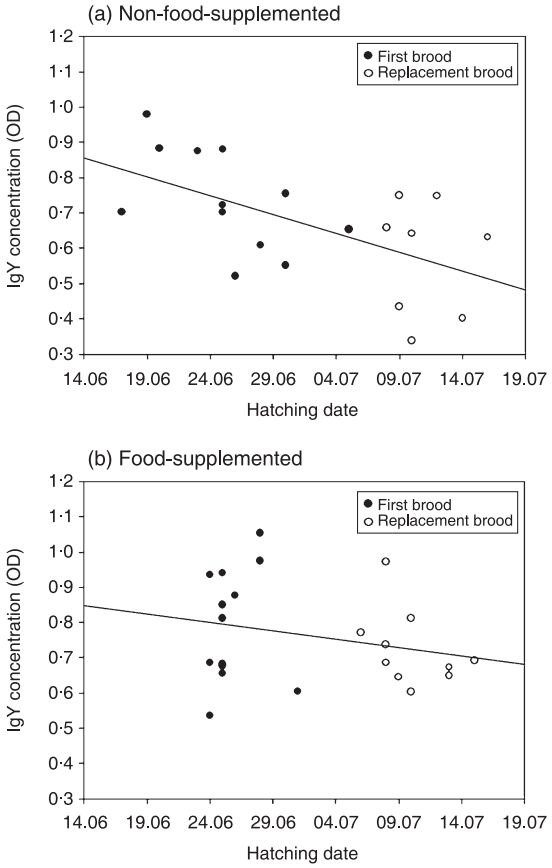

Fig. 1. Seasonal change in humoral immunocompetence (IgY concentration) in 10-day-old (a) non-food-supplemented and (b) food-supplemented chicks.

T-cell immunocompetence

The seasonal decrease in T-cell immunocompetence was not affected by the food treatment (full ANCOVA model with the interaction between hatching date and food treatment: $F_{1,36} = 0{\cdot}22$, $P = 0{\cdot}64$; Fig. 2). Because food supplementation did not improve T-cell immunocompetence (similar ANCOVA model without the interaction: $F_{1,37} = 0{\cdot}15$, $P = 0{\cdot}70$; hatching date: $F_{1,37} = 12{\cdot}07$, $P = 0{\cdot}001$), we cannot make a firm conclusion about the factors that caused the seasonal decline in T-cell immunocompetence. In both non-food-supplemented ($F_{1,15} = 5{\cdot}81$, $P = 0{\cdot}03$) and food-supplemented chicks ($F_{1,21} = 5{\cdot}74$, $P = 0{\cdot}03$), the T-cell immunocompetence was higher earlier in the season. Just as with the results from humoral immunocompetence, results were similar when we added clutch sequence (first or replacement) in the model (full ANCOVA: interaction between food treatment and hatching date: $F_{1,35} = 0{\cdot}00$, $P = 0{\cdot}99$; similar ANCOVA after having removed the interaction: food treatment: $F_{1,36} = 0{\cdot}30$, $P = 0{\cdot}59$; hatching date: $F_{1,36} = 10{\cdot}63$, $P = 0{\cdot}002$; clutch sequence: $F_{1,36} = 3{\cdot}60$, $P = 0{\cdot}07$). Similarly, the effect of hatching date on T-cell immunocompetence remained significant ($P < 0{\cdot}01$) when we added, in turn, clutch size, egg weight and total clutch weight or time of incubation as covariates in the ANCOVAs (all P-values > 0·05). Applying a non-parametric ANCOVA, as we did for humoral immunocompetence, we confirm an effect of hatching date on T-cell immunocompetence, but no effect of food supplementation (interaction between food supplementation and hatching date: $P = 0{\cdot}64$; after removing the interaction: food supplementation: $P = 0{\cdot}73$, hatching date: $P = 0{\cdot}004$).

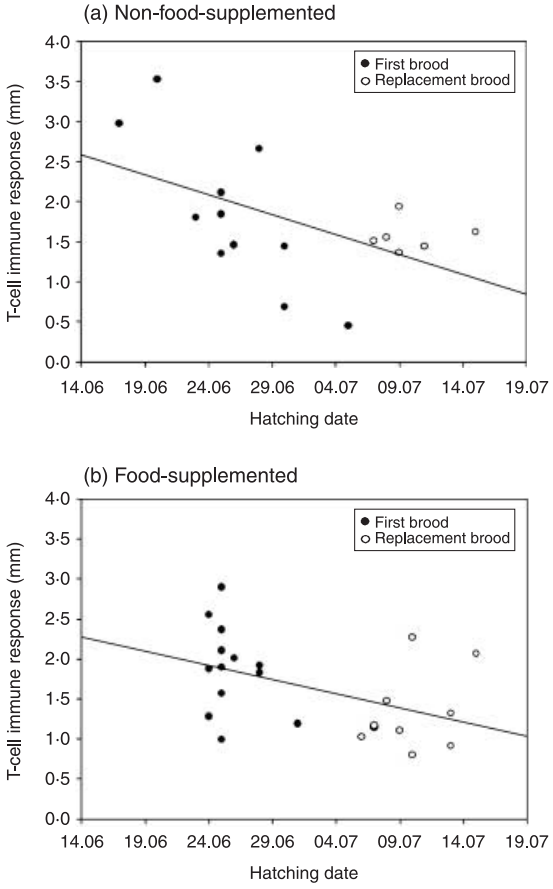

Fig. 2. Seasonal change in T-cell immunocompetence (PHA-response) in 20-day-old (a) non-food-supplemented and (b) food-supplemented chicks.

Discussion

This study investigated whether the seasonal decrease in chick immunocompetence is due to a decrease in food availability throughout the breeding season, as suggested by Sorci *et al*. (1997). We found evidence of this with the humoral, but not the T-cell immunocompetence. However, while food supplementation significantly increased humoral immunocompetence in 10-day-old chicks (IgY concentration), it did not entirely compensate for the seasonal decline observed in non-food-supplemented chicks (Fig. 1). This result provides experimental support to the hypothesis that food availability explains, at least in part, the seasonal decline in immunocompetence (Sorci *et al*. 1997).

There was no evidence that food supplementation enhanced T-cell immunocompetence, contrary to our findings with humoral immunocompetence. It should nevertheless be noted that individuals had more time to accumulate resources via food supplementation for late breeders (replacement broods) compared with early breeders (first broods). Given that feeding conditions have been shown to alter significantly the T-cell immunocompetence in two bird species, Barn Swallows (*Hirundo rustica*, Saino, Calza & Møller 1997) and Yellow-Legged Gulls (*Larus cachinnans*, Alonso-Alvarez & Tella 2001), the absence of a significant effect of food supplementation on T-cell immunocompetence in kittiwakes may be interpreted in two ways. First, food supplies were not different enough in non-food-supplemented and food-supplemented chicks. Even if the food supplementation experiment significantly altered humoral immunocompetence, indicating that we successfully created two groups of birds differing in the amount and/or quality of food they ingested throughout the breeding season, this difference was perhaps not pronounced enough to create two groups of birds that differ in T-cell immunocompetence. Indeed, some kittiwakes consumed very few Capelins, implying that nutritional status between the two food treatments was not large. Second, in contrast to Barn Swallows and Yellow-Legged Gulls, T-cell immunocompetence may be weakly sensitive to food supply in the kittiwake. This is possible because T-cell immunocompetence is typically positively correlated with body condition (Alonso-Alvarez & Tella 2001) but in our sample of nestling kittiwakes it was not the case (Pearson correlation between T-cell immunocompetence and residuals of the regression of body mass on wing size, $r = 0{\cdot}13$, $P = 0{\cdot}43$, $n = 40$). Therefore, it is possible

that T-cell immunocompetence is, in kittiwakes, sensitive to other temporal aspects of breeding, as hatching date had a much greater effect than the food treatment.

We can propose a couple of avenues of research to explain why T-cell and humoral immunocompetence could decrease with date in chicks independently of changes in the quantity of food available. First, food availability may vary not only quantitatively throughout the breeding season, but also qualitatively, which might explain seasonal variation in immunocompetence. Second, individuals of higher-quality commonly breed earlier in birds (e.g. Christians, Evanson & Aiken 2001), which could affect the seasonal variation in nestling immunocompetence via direct or indirect intergenerational effects. Further, independently of food supply, mothers may invest more resources that later improve chick T-cell and humoral immunocompetence (so-called maternal effects, e.g. Gasparini *et al*. 2001) in the first breeding attempt than in a possible replacement clutch (Gasparini *et al*. in press). This clutch-dependent pattern of maternal investment could be adaptive, because the likelihood of producing a replacement clutch is relatively low in kittiwakes (probability of failing at the first breeding attempt × probability to produce a replacement clutch if the first is lost = 21–32%; Wooller 1980; Barrett 1996; Gill & Hatch 2002). Moreover, in most birds, offspring reproductive value decreases with hatching date (Moreno 1998) and thus females may be selected to invest more resources in eggs laid early than in eggs laid later in the season.

In conclusion, this study show that the seasonal decline in chick humoral immunocompetence may be explained, at least in part, by the seasonal decline in food availability, as suggested by Sorci *et al*. (1997). However, alternative temporal factors may participate in synergy to this seasonal decline.

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