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Offspring body condition and immunocompetence are negatively affected by high breeding densities in a colonial seabird: a multiscale approach

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Why avian colonies vary in size and how food competition among nearby colonies affects offspring quality are still not completely understood. We simultaneously examined the effects of four scales of breeding density on two measures of offspring viability (body condition and T-cell-mediated immunity) in the colonial Magellanic penguin. Body condition of fledglings was inversely correlated with breeding density within 100 m^2 of nests, and decreased with increasing numbers of breeding pairs competing within the parental foraging ranges (100 km), probably as a result of density-dependent food depletion. The T-cell-mediated immune response was positively correlated with body condition, reflecting, to some extent, the previous breeding-density effects, and was negatively correlated with colony size, which may be related to social stress. However, given the effect of protein intake on cell immunity, this result could also indicate a thus far neglected cost of coloniality, namely the consumption of low-protein food to compensate for the depletion of optimal prey. These results were not influenced by other traits, nor by the current exposure of birds to parasites and diseases, as measured by serological variables. Since body condition and the T-cell-mediated immune response of fledgling birds are indicators of their survival and recruitment prospects, the costs we have identified can explain variability in colony size in relation to food competition with surrounding colonies, as well as the skewed distribution toward small colonies in this species.

Keywords: breeding density; coloniality; body condition; immunocompetence; Magellanic penguin

1. INTRODUCTION

Coloniality is a common breeding strategy in birds (Brown & Brown 1996). In recent decades much effort has been devoted to identifying ecological and evolutionary factors that may explain the origin and function of avian coloniality, resulting in considerable debate (Brown & Brown 1996; Danchin & Wagner 1997; Tella *et al*. 1998). However, relatively little research has been directed at explaining why colonies vary in size and at identifying factors that may limit their growth (Brown *et al*. 1990). Whatever the origin of coloniality, high breeding densities may impose costs in terms of increased transmission of pathogens and increased competition for food, potentially resulting in reduced offspring condition. In fact, some studies have revealed a lower body mass of fledglings with higher breeding densities around the nest (Phillips *et al*. 1998) and larger colony size (reviewed in Brown & Brown 1996). Since chick fledging weights are positively correlated with juvenile survival in a number of species (Magrath 1991), large breeding densities may impose long-term fitness costs not measured by immediate breeding success, and these costs could limit the size of large colonies by reducing recruitment rates.

Previous studies showing relationships between fledgling body mass and breeding density have compared breeding patches within colonies or among colonies differing in the number of breeders (e.g. Brown & Brown 1996; Phillips *et al*. 1998), without considering potential inter-colony effects. However, there is increasing evidence for food competition between members of neighbouring colonies. Food depletion around a colony may depend not only on its size but also on the size of the conspecific population breeding around it. This may account for why the number of competitors from surrounding colonies is an important predictor of colony size in some marine (Furness & Birkhead 1984) and terrestrial (Griffin & Thomas 2000) species.

In this study, we examined the combined effects of breeding density measured at four spatial scales (breeding density around the nest, mean breeding density in the colony, colony size and breeding density around the colony) on offspring quality in a colonial species, the Magellanic penguin (*Spheniscus magellanicus*). This seabird breeds at highly variable densities (Yorio *et al*. 1998) and is thus a good model for testing the effects of both local breeding densities and competition with nearest colonies on different measures of offspring quality, an approach that, to our knowledge, has never been considered in avian studies. As a traditional measure of offspring viability we used the body condition (i.e. body mass corrected

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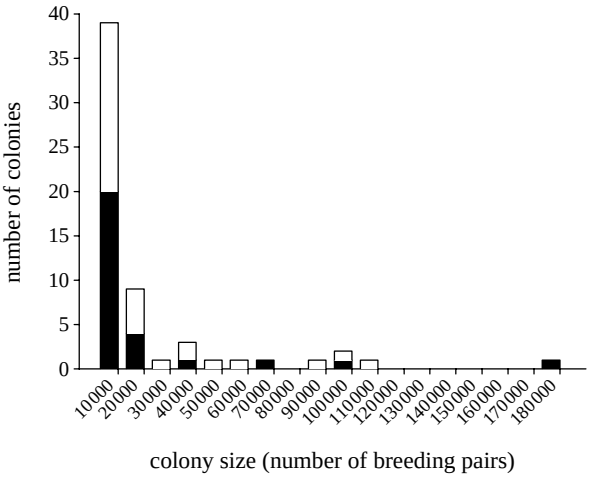

Figure 1. Frequency distribution of the size of Magellanic penguin colonies on the Atlantic coast of South America. The black portions of the bars indicate our study area, Chubut Province, Argentina.

for size; e.g. Merilä *et al.* 1999) of fledglings. We also measured the T-cell-mediated immunity (CMI) of fledglings, which is one of the three main components of immunocompetence in vertebrates (Norris & Evans 2000). CMI is an important factor determining resistance to intracellular infections (viral, bacterial, protozoal and fungal), delayed hypersensitivity and tumour rejection (Bowry 1984). Recent studies have shown relationships between avian CMI and nutritional condition (reviewed in Alonso-Alvarez & Tella 2001) as well as other components of fitness in birds (Norris & Evans 2000), including probability of survival (González *et al.* 1999; Horak *et al.* 1999; Soler *et al.* 1999; Merino *et al.* 2000; Tella *et al.* 2000*a*). In fact, CMI seems to be a better predictor of juvenile survival and recruitment than body condition in some species (Merino *et al.* 2000; Tella *et al.* 2000*a*). We measured leucocyte differentials to control for the current exposure of birds to a potentially wide array of parasites and diseases (Campbell 1994; Clark & Kerry 2000). We predicted that if high breeding densities impose fitness costs due to density-dependent food depletion then these costs should be reflected in reduced body condition and/or poorer immunocompetence of fledglings, and the magnitude of these costs should vary depending on the scale of breeding density measured.

2. METHODS

(a) *Distribution of colony sizes and study area*

A total of 59 colonies of Magellanic penguins are distributed along the Atlantic coast of South America (Yorio *et al.* 1998). This species shows a skewed distribution of colony sizes, with most colonies (66%) holding fewer than 10 000 pairs, and only two with more than 100 000 pairs (figure 1). We conducted this study in Chubut Province, Argentina, where there are 28 colonies (Yorio *et al.* 1998). Variability in colony size within this area closely resembles that for the whole area of distribution (figure 1). We selected six colonies distributed from Península Valdés (42°04′ S, 63°1′W) to Cabo Dos Bahías (44°54′ S, 65°32′ W). The size of these colonies ranged from 483 to 175 000 breeding pairs, and the most distant colonies were 340 km apart. Therefore, our sampling covered known variability in colony size, and we minimized the potential influences of variations in food supply between areas of differing productivity, which could mask density-dependent food-depletion effects in seabirds (Furness & Birkhead 1984).

(b) *Offspring body condition and immunocompetence*

During January and February 1999, we selected nests balanced for local breeding density, brood size and other factors potentially affecting body condition and CMI (see § 2c). Chicks were captured while attended by their parents (usually just before being fed) and nests were marked for easier location 24 h later. Bill and flipper lengths were measured during the first visit and weights were obtained on both visits (range, 1125–3850 g). Sex was determined by molecular procedures (Ellegren 1996) using DNA extracted from blood, and age was estimated from bill length (age in days $= -56.76 + 2.98 \times$ bill length, $r^2 = 0.98$, $p < 0.001$; Boersma *et al.* 1990, fig. 1.7) since the bill grows linearly with age and is relatively unaffected by inter-year variations in food supply (Boersma *et al.* 1990). Chicks were sampled a few days before they were due to fledge (age of 68 ± 6.8 days, $n = 315$).

Since fledgling body mass may vary between days depending on feeding rates (Williams 1995), we used the lower of the two weights obtained on consecutive days to reduce the potential effect of recently ingested food on body mass. Body mass was also influenced by age and size (as indicated by bill and flipper lengths) and the sex of fledglings (Forero *et al.* 2001). Therefore, we obtained, as an index of body condition, the residuals from an analysis of covariance (ANCOVA) with log body mass as the dependent variable, sex as a factor and log-transformed flipper and bill lengths as covariates ($r^2 = 0.38$, $F_{3,314} = 63.07$, $p < 0.001$).

We measured CMI by challenging the immune system through the subcutaneous injection of a novel mitogen (phytohaemagglutinin), which produces a prominent perivascular accumulation of T lymphocytes followed by macrophage infiltration (Smits *et al.* 1999). This technique has been shown to be a reliable indicator of *in vivo* cellular immunity in birds, and it is being increasingly used in ecological studies (see § 1). We injected 0.1 ml of 2 mg ml^{-1} phytohaemagglutinin (Sigma, St Louis, MO, USA) in phosphate buffered saline into a marked site on the right external foot web (Moreno *et al.* 1998). The thickness of the foot web was measured with a micrometer (± 0.001 mm) at the injection site three times, just prior to and 24 h (± 15 min) after injection. Since the repeatability of the three measurements was high for a random sample of 30 fledglings ($r = 0.99$, $F_{29,89} = 59\,765.1$, $p < 0.001$), the CMI response was calculated as the mean change in thickness between the day of injection and the following day (Smits *et al.* 1999). To determine leucocyte differentials, we smeared a drop of blood on a microscope slide, which was then air-dried, fixed in absolute ethanol and later stained with Giemsa stain. The proportions of heterophils, lymphocytes, eosinophils and monocytes, and the heterophil-to-lymphocyte ratio were determined by identifying 100 cells under 1000 power in fields selected in an evenly distributed monolayer (Moreno *et al.* 1998; Tella *et al.* 2000*a*).

(c) *Breeding densities and other variables*

We defined four scales of breeding density. Local breeding density was defined as the number of nests in circular plots of 100 m^2 centred on each sampled nest (range, 1–55 nests per

100 m^2). Sampled nests were spaced so that no circular plots overlapped. Mean colony density was defined as the total number of nests divided by the area covered by the colony (from Yorio *et al*. 1998) and ranged from 33 to 644 nests ha^{-1}. Colony sizes were 483 breeding pairs in Asentamiento Oeste, 1553 breeding pairs in Caleta Interna, 9067 breeding pairs in Cabo Dos Bahías, 13 780 breeding pairs in Isla Primera, 17 034 breeding pairs in San Lorenzo and 175 000 breeding pairs in Punta Tombo (Yorio *et al*. 1998). Population breeding density was defined as the number of breeding pairs (Yorio *et al*. 1998) potentially competing for food at sea within circles centred on each sampled colony (i.e. the number of breeding pairs in the sampled colony plus the number of breeding pairs from other colonies corrected for the area of overlap of their circles, excluding the area covered by land). Breeding Magellanic penguins in our study area forage at a mean distance from the colony of 117 km (range, 0–303 km; Wilson *et al*. 1995). We calculated population breeding densities around each sampled colony using circles of 30 km, 100 km, 150 km, 200 km, 250 km and 300 km radius. Since these densities were highly correlated, we used only the 100 km radius (the closest to the mean foraging distance) in our analyses. The assumptions made were that all colonies extend their feeding ranges to similar distances and that birds are equally likely to forage in all parts of this range (Furness & Birkhead 1984; Griffin & Thomas 2000). However, mean oceanic productivity in temperate regions may not be as uniform as believed (Furness & Birkhead 1984), and colony foraging ranges may be irregularly shaped or unevenly used (Cairns 1989). However, these potential biases should simply increase statistical noise and reduce the strength of relationships (Furness & Birkhead 1984; Griffin & Thomas 2000).

Body condition and the CMI of fledglings could also be influenced by some individual- and nest-related traits. To control for these sources of variability, we measured nine other explanatory variables. Among individual traits, we considered age and sex for CMI analysis, and laying date (by allowing for 41 days of incubation plus the age of the chick on the day it was sampled), brood size and within-brood size hierarchy (single chick, larger and smaller chick in double broods) for both body-condition and CMI analyses. These variables have been shown to affect body condition and/or CMI in birds (e.g. Moreno *et al*. 1994; Saino *et al*. 1997; Sorci *et al*. 1997). We also measured three variables relating to nest-site characteristics: type of nest (under bushes, in burrow, or in burrow under bushes), nest cover (estimated visually to the nearest 10% from a position directly above the nest; range, 0–100%) and nest depth (distance from the nest entrance to the end of the nest-cup chamber; range, 0–313 cm). These characteristics could determine differences in microclimate conditions in the nests (García *et al*. 2001), which may affect heat stress in chicks and their CMI and overall condition (Regnier & Kelley 1981). Given that some fledglings leave the nest when not attended by their parents, we also estimated the cover of bushes (to the nearest 10% in circular plots of 100 m^2 centred on each sampled nest; range, 0–100%) as a measure of the shade availability for the fledglings.

(d) *Statistical analyses*

We performed multivariate analyses to identify the effects of breeding aggregations on body condition and CMI of fledglings while controlling for other confounding factors. Since fledgling body condition and CMI are influenced by common rearing environments (Brinkhof *et al*. 1999; Merilä *et al*. 1999; Tella *et al*. 2000*b*), we considered that chicks from the same nest and colony were not independent samples. Therefore, nests and colonies were treated as random effects using a randomized block design, in which individuals were 'nested' on their nests and each nest was 'nested' on six different blocks that were colonies. By doing so, we controlled for individual traits in the analyses while avoiding pseudoreplication. An additional benefit of random effects is that they allow inferences to be made about the significant fixed effects that can be applied to the whole population, not only to the data set analysed. We conducted separate analyses for body condition and CMI as response variables. Body condition was also considered as an explanatory variable for the CMI analysis because of its influence on this aspect of immunocompetence (Alonso-Alvarez & Tella 2001). Since leucocyte differentials and the heterophil-to-lymphocyte ratio reflect the exposure of individuals to a wide array of parasites and diseases (Campbell 1994; Clark & Kerry 2000), they were used as explanatory variables for both body condition and CMI. We fitted all the explanatory variables to the observed data

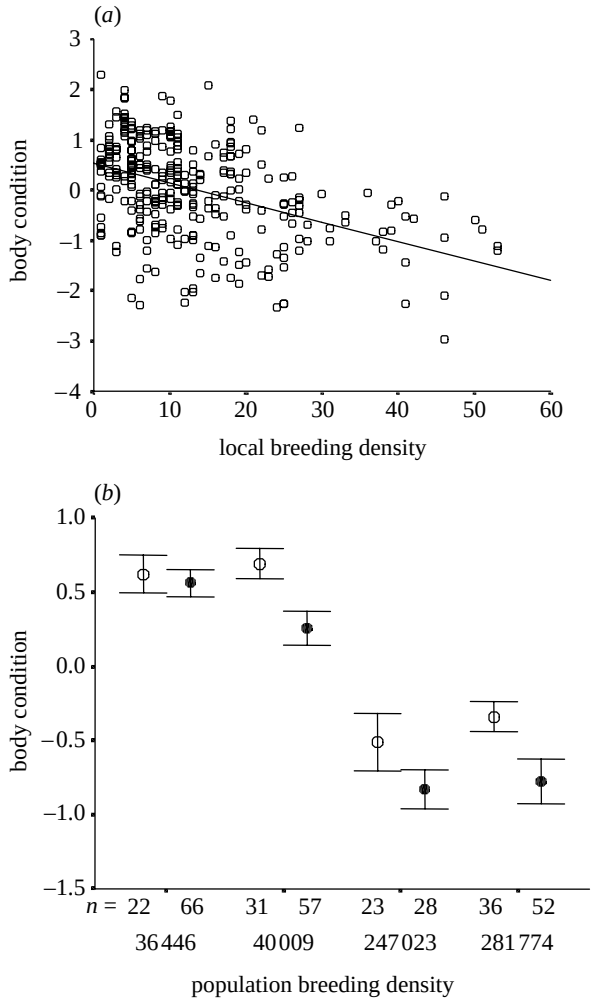

Figure 2. Relationships between the body condition of fledglings and (*a*) the local breeding density (100 m^2 around the nest) and (*b*) the population breeding density (radius of 100 km), where means and standard deviations for one- (open circles) and two-chick (closed circles) broods are represented, and sample sizes (*n*) are given above the population breeding densities. Two out of the six colonies have identical population breeding densities. Note that these are univariate trends (see § 3 for multivariate tests).

using generalized linear mixed models (GLMM), which allow the distinction between fixed and random effects (Littell *et al.* 1996). Since both CMI and body condition were normally distributed, we used the normal error and identity link function in the macro GLIMMIX of SAS (Littell *et al.* 1996). Some of the explanatory variables could covary, so we fitted their effects to the observed data following a forward stepwise procedure, testing the significance of each variable and adding only the variable that resulted in a better fit to the model. The significances of the remaining variables were tested again until no additional variable or interaction reached significance. The result is the most adequate model for explaining the variability in the response variable, where only the significant explanatory variables are retained. All tests are two-tailed.

3. RESULTS

We measured the body condition and CMI of 315 fledglings in 222 nests from six colonies. The GLMM for body condition showed that the variability was explained by lymphocytes ($F_{2,96}=12.14$, $p=0.0008$), brood size ($F_{1,96}=6.19$, $p=0.01$), local breeding density ($F_{1,96}=10.83$, $p=0.001$) and population breeding density ($F_{1,96}=34.04$, $p=0.0001$), after controlling for birds sharing nests ($\chi=2.77$, $p=0.005$) and colonies ($\chi=0.79$, $p=0.43$). This model explained 74% of the original deviance. Figure 2 shows the univariate trends for these sources of variability. The body condition of fledglings was poorer at higher local breeding densities (figure 2*a*), in fledglings belonging to double broods and in those raised in high population breeding densities (figure 2*b*). The relationship between body condition and lymphocytes was positive (plot not shown).

The GLMM obtained for CMI revealed that it was related to body condition ($F_{1,96}=13.09$, $p=0.0005$), lymphocytes ($F_{2,96}=4.07$, $p=0.04$), within-brood size hierarchy ($F_{2,96}=4.70$, $p=0.01$) and colony size ($F_{1,96}=7.59$, $p=0.007$), after controlling for birds sharing nests ($\chi=4.09$, $p=0.0001$) and colonies ($\chi=0.70$, $p=0.48$). CMI was positively correlated with body condition (figure 3*a*) and lymphocytes, and negatively correlated with colony size (figure 3*b*). CMI also differed between single and between larger and smaller fledglings of double broods, with smaller siblings tending to have a lower CMI (figure 3*b*). This model explained 77.4% of the original deviance.

4. DISCUSSION

Some brood-related traits explained variability in the body condition and CMI of fledgling Magellanic penguins. Fledglings from broods of two were in poorer body condition than individuals raised singly. This finding reflects the classical trade-off between the number and the viability of progeny at independence (e.g. Tella *et al.* 2000*a*) and results from food limitation, as found in other penguin species (Moreno *et al.* 1994). On the other hand, CMI was influenced by the within-brood hierarchy of fledglings. This may be because the larger chick gets most of the food provided by the parents (Blanco *et al.* 1996); also, the maternal deposition of physiologically active compounds that enhance the immunocompetence of the chicks (Graczyk *et al.* 1994; Haq *et al.* 1996) may be differentially allocated between eggs according to laying sequence (Royle *et al.* 1999). Once these factors were taken into account in multivariate analyses, different measures of breeding density explained the most variability (as indicated by F values) in body condition and CMI. Body condition was poorer at higher local and population breeding densities, while CMI was inversely related to colony size. Since both nest and colony identities were fitted as random effects, these results can be generalized to the whole population (Littell *et al.* 1996).

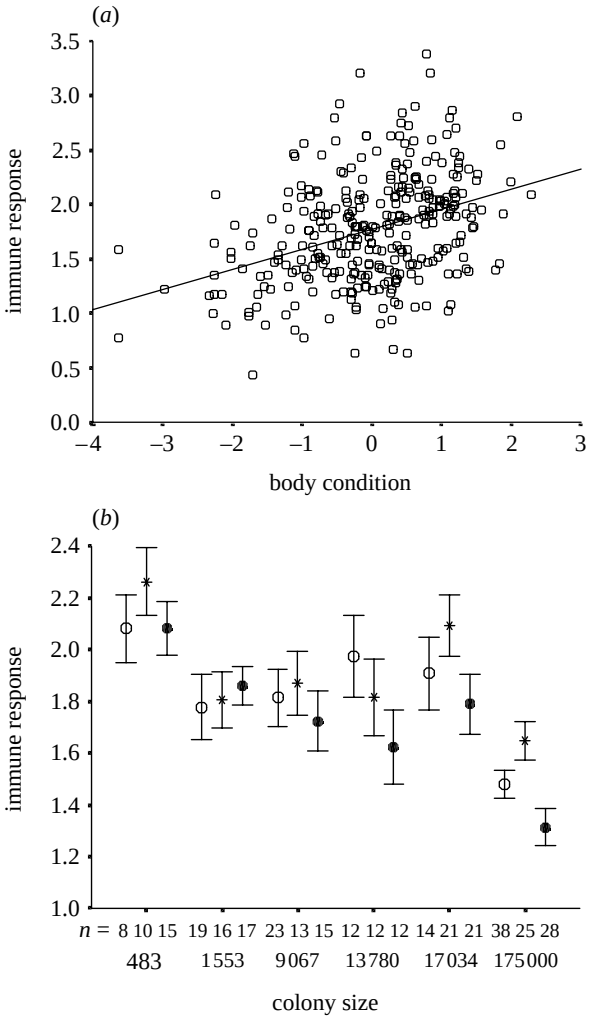

Figure 3. Relationships between T-cell-mediated immune response of fledglings and (*a*) their body condition and (*b*) their colony size (number of breeding pairs), where means and standard deviations for single chicks (open circles) and larger (asterisks) and smaller (closed circles) chicks from double broods are represented, and sample sizes (n) are given above the colony sizes. Note these are univariate trends (see § 3 for multivariate tests).

The fact that large breeding aggregations negatively affect offspring quality could be explained both by the effects of parasites and by conspecific food competition. Parasites are known to detrimentally affect the body condition of fledglings in many bird species (e.g. Merino & Potti 1995), and parasite loads may increase with increasing degrees of host aggregation in colonial birds (Brown & Brown 1996). Moreover, large breeding aggregations could indirectly reduce the offspring CMI through its connection with body condition, or because

parasites challenge other parts of the immune system specifically, causing nestlings to invest less in CMI (Brinkhof *et al.* 1999). Although we did not find haemoparasites in any of the sampled penguins (Jovani *et al.* 2001), and ectoparasites infected only 12%, with an extremely low abundance per bird (which did not affect the body condition or the CMI of fledglings; J. L. Tella *et al.*, unpublished data), we cannot rule out the presence of other pathogens of penguins usually undetected in field surveys (Clark & Kerry 2000). However, the fact that the effects of breeding densities on body condition and CMI remain highly significant after controlling for leucocyte profiles, which reflect the current exposure of birds to a variety of infections (Campbell 1994; Clark & Kerry 2000), indicates that our results reflect factors other than diseases. So, the negative relationship that we found between local breeding density and body condition is unrelated to potential diseases favoured by crowded conditions (Clark & Kerry 2000). Stokes & Boersma (2000) found that local breeding density (also measured in 100 m^2 plots) in one of our sampled colonies was inversely correlated with breeding success, due to higher rates of conspecific fighting and predation in high-density plots. Therefore, adults breeding at high local densities would be exhausted at the end of the breeding cycle (which extends for more than 5 months) because of a higher investment in nest defence, thus devoting less time to food provisioning, and raising fledglings in poorer condition (see also Phillips *et al.* 1998).

Theoretical and empirical studies have shown that increasing conspecific density may result in food depletion and, consequently, in a density-dependent reduction in breeding output (Sutherland 1996). Evidence for food depletion in colonial birds comes from studies showing lower body masses of fledglings in larger colonies (but see Brown & Brown 1996). However, the potential role of competition with individuals breeding in neighbouring colonies has been poorly examined (Hunt *et al.* 1986). We found that most of the variability in fledgling body condition was explained by the population breeding density in the parental foraging range; the average body condition was *ca.* 10 units higher in two areas of low density than in two areas holding much larger densities (figure 2*b*). This suggests that density-dependent food depletion acts at a larger scale than the local colony, and supports the hypothesis that there is inter-colony competition for food in colonial seabirds (Furness & Birkhead 1984; Forbes *et al.* 2000). Such food competition would also indirectly affect the CMI of fledglings because this was partially influenced by body condition (figure 3*a*), as a result of the well-known role of feeding rates (Saino *et al.* 1997) and the resulting body condition of fledglings on their CMI (Alonso-Alvarez & Tella 2001). Furthermore, CMI was negatively correlated with colony size, an effect that was more marked in the smaller fledglings of double broods (figure 3*b*). Since social stress increases the level of circulating corticosteroids, which in turn affect the immune system (Braude *et al.* 1999), large colonies may constitute stressful environments for chicks and may thus compromise their CMIs, especially in smaller chicks, which may suffer the additional stress of having to compete with stronger siblings. Although the heterophil-to-lymphocyte ratio, as a measure of stress in penguins (Vleck *et al.* 2000), did not enter significantly ($p = 0.08$) into our GLMM for CMI, we cannot completely discard this possibility, given the still poorly understood relationship between stress and immunoredistribution in vertebrates (Braude *et al.* 1999). On the other hand, recent experiments have shown that the CMI is not only related to food intake but also to the amount of proteins ingested during growth (Saino *et al.* 1997; Birkhead *et al.* 1999; González *et al.* 1999). Dietary studies conducted in our study population showed a considerable variability between nearby colonies in the kind of prey consumed, which differed in caloric and protein contents and which could be related to conspecific food competition rather than to natural variability in food supplies (J. L. Tella *et al.*, unpublished data). Therefore, large colonies may impose a cost to parents through the acquisition of low-protein prey to compensate for a depletion of the optimal prey, thus resulting in a poorer offspring immunocompetence.

As far back as 1963, Ashmole proposed that seabird populations are limited by density-dependent competition for food around their breeding colonies. There is evidence to suggest that seabirds may deplete food supplies around the colony (Birt *et al.* 1987) and that colony sizes vary inversely with the number of conspecifics breeding within their potential foraging ranges (Furness & Birkhead 1984; Forbes *et al.* 2000; see also Griffin & Thomas (2000) for a non-seabird species), thus giving support to Ashmole's (1963) hypothesis. However, the proximate mechanisms for colony- and population-size regulation are not well known. Given that Magellanic penguins, as well as other penguin species, tend to be highly faithful to their natal colonies (Williams 1995), differences in offspring viability between colonies could explain variability in colony size through differential survival and local recruitment rates. We have shown here that high breeding densities, measured at different spatial scales, impose costs in terms of two measures of offspring quality closely related to survival prospects. High local and population breeding densities result in a reduction in offspring body condition and compromise at least one important aspect of offspring immunocompetence. These costs can satisfactorily explain the variability in colony size related to food competition with surrounding colonies, as well as the skewed distribution towards small colonies in this species (figure 1). Unfortunately, to our knowledge, there are no data available on the long-term recruitment rates of banded fledglings or the colony-size dynamics in relation to differing breeding densities in this or any other penguin species. However, there is circumstantial evidence gathered in our study area that supports our predictions. The largest colony of Magellanic penguins (Punta Tombo), which is located in a high-population-size area, has reduced in size by over 20% in the last two decades, while the medium-sized colony of Cabo Dos Bahías (also in an area of high population breeding density) has decreased only slightly, and the small- to medium-sized colonies of Península Valdés (sited in an area of low population breeding density) have increased in size over the same period (Capurro *et al.* 1988; Carribero *et al.* 1995; Williams 1995; Yorio *et al.* 1998).

To conclude, our results suggest that different scales of breeding density should be considered in order to

identify correctly the costs of coloniality on offspring fitness and to understand the degree of inter-colony competition and the population dynamics of pelagic seabirds. In particular, immunocompetence, an important component of fitness, which until recently was not considered in evolutionary ecology (Norris & Evans 2000), offers potential insights into the still poorly understood mechanisms behind the extensive variation in colony size in birds (Brown *et al*. 1990).

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