

Habitat-specific effects of a food supplementation experiment on immunocompetence in Eurasian Magpie *Pica pica* nestlings

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Parasite pressure and nutrition are two of the most important factors affecting the trade-off between nestling growth and immune development. During development, energy and nutrients are often limited, and nestlings should only dedicate differentially more valuable resources to their immune system when the associated benefits are high (i.e. in situations of an increased risk of parasitism). In this study, we manipulated nutritional condition of Eurasian Magpie *Pica pica* nestlings by providing a food supplement. Additionally, the study area was subdivided into two categories of habitat (irrigated and arid) based on the presence of irrigation canals. Nestling diet composition was more varied in the irrigated compared with in the arid habitat. In addition, nestlings of the irrigated habitat showed a significantly higher infestation of both ectoparasites and blood parasites and a significantly higher cell-mediated immunity, but lower tarsus length compared with nestlings of the arid habitat. Food supplementation to nestlings did not affect tarsus length, but increased nestling cell-mediated immunity in the arid habitat only. Based on the recent demonstrated trade-off between growth and immunocompetence in nestlings, we suggest that differences between habitats in nestling diet and parasite prevalence may have caused different priority rules in the allocation of resources between both fitness traits.

The immune system is the most important physiological means allowing animals to fight or control parasitic or pathogenic infections. Mounting an immune response as well as maintaining a competent immune system is expensive (Råberg *et al.* 2000, Eraud *et al.* 2005), so investments in immune defence should be adjusted not only according to the availability of resources (Sheldon & Verhulst 1996, Lochmiller & Deerenberg 2000, Norris & Evans 2000), but also according to the risk of parasitism. This is because in the hypothetical extreme case of a parasite-free environment, any immune investment would be pointless (Zuk & Stoehr 2002, Schmid-Hempel & Ebert 2003). In this context, numerous studies have suggested that parasites mediate important host life-history trade-offs (e.g. Sheldon & Verhulst 1996,

Coustau *et al.* 2000, Lochmiller & Deerenberg 2000, Norris & Evans 2000, Zuk & Stoehr 2002). Individuals living in high pathogenic environments should increase their investment in immune response (Piersma 1997, Lochmiller & Deerenberg 2000). Indeed, a relationship between immunocompetence of adult birds and variables indicative of the abundance of parasites has been demonstrated both at the interspecific (Møller 1997, Piersma 1997, Møller & Erritzøe 1998, Martin *et al.* 2001, Møller *et al.* 2003) and at the intraspecific level (Lindström *et al.* 2004).

Trade-offs between immunocompetence and other life-history traits would be particularly important during development because energy and nutrients required for growth are often limited (Lochmiller & Deerenberg 2000). In fact, a trade-off between growth and immunity in bird populations has been suggested several times (Saino *et al.* 1998, Merino *et al.* 2000a) and was recently experimentally confirmed

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in a field population of Eurasian Magpies *Pica pica* (Soler *et al.* 2003) and Blue Tits *Cyanistes caeruleus* (Brommer 2004). Furthermore, this trade-off may become especially relevant to life-history theory when variation in the pathogenicity of rearing environments is taken into consideration (Lochmiller & Deerenberg 2000). Given the high energetic requirements of the growth process, developing nestlings should only dedicate differentially more valuable resources to their immune system when the associated benefits are higher. As ecto- and haemoparasite infestations may cause detrimental effects on nestlings (Richner *et al.* 1993, Hurtrez-Boussès *et al.* 1997, Merino *et al.* 2000b), the allocation of resources to the development of the immune function should be positively related to the risk of being parasitized (Piersma 1997, Lochmiller & Deerenberg 2000, Merino *et al.* 2000a). However, this allocation of resources to immune function will also depend on nutritional conditions and therefore detrimental effects of parasites may be accentuated in situations of food scarcity (de Lope *et al.* 1993, Merino & Potti 1998, Appleby *et al.* 1999, Blanco *et al.* 2001, Simon *et al.* 2004). Thus, the nutritional condition of nestlings may limit investment in immunity, but may also influence the balance between costs and benefits associated with a differential investment in immunity during growth.

In the present study, we manipulated nutritional conditions of nestlings in a Magpie population by providing a food supplement to half of the nestlings in each nest. Additionally, we subdivided our study area into two categories of habitat, based on the presence of irrigation canals (irrigated and arid habitat), which were expected to differ in food availability and/or parasite prevalence. Many parasites have complicated life cycles and some free-living stages may be vulnerable to specific environmental conditions restricting their distribution. Currently, evidence exists of parasite loads in birds varying between different climatic zones and habitats (Tella *et al.* 1999). For example, individuals living in humid habitats have been found to contain heavier parasite loads compared with individuals living in arid habitats (Van Riper 1991, Dobson *et al.* 1992, Moyer *et al.* 2002), and therefore we expect the arid habitat to be a less pathogenic environment (nestling infestation) compared with the irrigated area. In addition, because food availability and nestling diet may change in relation to habitat characteristics (Christe *et al.* 1996, Tripet & Richner 1997, Johnson & Lombardo 2000), we evaluated nestling diet in both habitat types by

means of neck-collar trials. Furthermore, we analysed the effect of the food supplementation experiment on nestling body size, condition, immunocompetence and parasite infestation, and possible interactions with habitat, because the effect of the experimental food supplementation may differ depending on food availability in different habitats. We discuss results with respect to parasite prevalence, nestling diet and parental quality measurements (laying date, egg size, clutch size, reproductive success and nest size; Soler *et al.* 2001, De Neve *et al.* 2004).

METHODS

Study area and habitat structure

The experiments were performed during the springs of 2001 (food supplementation experiment), 2002 and 2003 (neck-collar trials) in a population of Eurasian Magpies breeding in the Hoya de Guadix (37°18'N, 3°11'W). This region is located in southern Spain and is a high-altitude plateau, approximately 1000 m asl. The vegetation includes cultivated cereals (especially barley), fallow and bare land, and many groves of almond trees *Prunus dulcis* in which Magpies prefer to build their nest (for a more detailed description, see Soler 1990).

The study area was divided into two categories of habitat, based on the presence of irrigation canals (Soler *et al.* 1998b). (1) Irrigated habitat: an extensive irrigation system characterizes the three villages included in the study area, which are predominantly surrounded by cultivated fields and groves of almond trees. Mature almond trees and a rich vegetation of grass and weeds border the fields and occupy the groves. (2) Arid habitat: where the irrigation network stops, the landscape becomes very barren, characterized by fallow and bare land with very sparse vegetation alternating with scattered, principally young, almond trees. The two areas are divided by a highway. Between the highway and the limit of the irrigation system surrounding the villages, there is a border of at least 2 km with very bare land and hardly any trees that was largely unused by Magpies, and thus the two populations were spatially separate.

A previous study found that Magpie nest density was markedly higher in the irrigated habitat (Soler *et al.* 1998).

During spring 2001, we measured nest size, laying date, clutch size, egg size and reproductive success to evaluate possible differences in parental quality between habitats. We visited Magpie nests no less

than twice a week to record laying date, and at least every 2 days during the laying period. After clutch completion, we measured the largest and shortest diameter of the eggs with a digital calliper (Mitutoyo, 0.01-mm accuracy), and nest size with a ruler (0.5-cm accuracy). Egg size and nest size were calculated as the volume of an ellipsoid: $4/3\pi ab^2$, where a is the largest radius and b the shortest (Soler *et al.* 2001).

Food supplementation experiment

After the 18th day of incubation, nests were visited daily to record hatching date. We provided food supplements to half of the nestlings in each nest during the nestling period. Two days after the first nestling hatched, each hatchling was weighed and marked with a unique colour on the tarsus. Subsequently, hatchlings were ranked according to their weight. Starting with the heaviest or second heaviest hatchling (alternating between nests), we assigned the food treatment to half of the hatchlings, alternating according to their weight. Brood size varied from two to eight hatchlings (mean 5.15 ± 0.18 se). The supplemental food consisted of a high-calorie dense paste enriched with essential micronutrients (minerals, vitamins, and amino acids; 5 calories/g; Nutri-Calorías, Shering-Plough Animal Health, used as a strong calorie and nutritional supplement for dogs and cats). The dose and frequency of the food treatment were calculated based on the product instructions for the mean weight of Magpie nestlings at 8 days of age (50 g) and consisted of 0.1 mL of the food (administered with a syringe). We revisited nests subsequently every 2 days, re-coloured the tarsi of all nestlings and fed the nestlings that were assigned to the treatment during the first visit (seven times during the nestling period). Nestlings easily swallowed the supplemental food, and we did not observe any adverse effects of the experiment on nestlings.

Body condition, immunocompetence and parasite prevalence

About 4–5 days before fledging, when the oldest nestling in the nest was 16–17 days old, we ringed, measured tarsus length and weighed all nestlings in the nest. At the same time, all nestlings were visually checked for ectoparasites. Magpie nestlings are mainly parasitized by *Carnus haemapterus* (Diptera: Carnidae; Soler *et al.* 1998). Marks of this ectoparasite are frequently found in the axillae of the wings of nestlings (adult flies lose their wings after reaching a host).

Furthermore, Magpie nestlings also showed conspicuous blood marks on their bellies, which were not related to the presence of *C. haemapterus* marks in the axillae (GLMMIX $F_{1,115} = 0.50$, $P = 0.48$, $n = 131$). This lack of relationship may suggest that other ectoparasites also infested Magpie nestlings, and therefore the observed ectoparasite marks were attributed to *C. haemapterus* (axillae) and ectoparasite sp. (belly). On each nestling, we noted the presence or absence of marks and estimated the severity of infestation based on these traces on each wing axilla and the belly, using a scale from 0.5 (little infected) to 4 (heavily infected; scale step 0.5), hereafter termed 'intensity of infestation'. We calculated the mean of both axillae (*C. haemapterus* intensity of infestation). When only infested nests were considered, mean nest infestation of *C. haemapterus* and ectoparasite sp. showed a normal distribution (Kolmogorov–Smirnov > 0.2). However, when considering individual nestlings, these variables did not meet a normal distribution. Therefore, when testing for intensity of infestation within each nest, we checked the residuals of the model for a normal distribution.

One blood smear was taken from the brachial vein of each nestling. Smears were subsequently air-dried, fixed in absolute ethanol for 3 min and dyed with Giemsa stain. Blood smears were then screened for blood parasites (*Haemoproteus* spp., *Leucocytozoon* spp. and *Trypanosoma* spp.). As parasitism prevalence was low (10.75%, $n = 214$ nestlings), we used parasite prevalence (i.e. presence or absence of blood parasites) in our analyses.

Finally, a phytohemagglutinin (PHA-P, Sigma Chemical Co.) injection was used to evaluate the *in vivo* cell-mediated immune response of nestlings (Cheng & Lamont 1988). We injected nestlings subcutaneously in the right wing web with 0.5 mg of PHA dissolved in 0.1 mL of physiological saline solution. The left wing web was injected with 0.1 mL of physiological saline solution. We measured the thickness of each wing web at the injection site with a digital pressure-sensitive micrometer (Mitutoyo, model ID-CI012 BS; to the nearest 0.01 mm) before and 24 h after the injection. The cell-mediated immune response (CMI) or wing web index was then estimated as the change in thickness of the right wing web (PHA injection) minus the change in thickness of the left wing web (Lochmiller *et al.* 1993). We repeated measurements of each wing web three times, and the mean was used in subsequent analyses.

We found a total of 168 first clutches, but subsequent sample sizes for clutch size, nest size and egg size

Table 1. Comparison of reproductive values (mean \pm se) between arid and irrigated habitats (spring 2001). Laying date, clutch size, egg size, nest size and the number of fledglings were analysed with GLM with laying date as a covariable (F -tests). Differences in hatching success, nestling mortality and fledging success were analysed using Mann–Whitney U -tests. Arid habitat $n = 30$, irrigated habitat $n = 28$.

	Arid habitat	Irrigated habitat	df error	F	P
Laying date (1 = 1 April)	21.5 \pm 1.0	17.3 \pm 1.1	167	8.3	0.0044
Clutch size	6.7 \pm 0.1	6.4 \pm 0.1	155	6.2	0.013
Egg size (mm ³)	95.9 \pm 1.1	96.5 \pm 1.1	85	0.16	0.68
Nest size (cm ³)	156.1 \pm 7.7	155.8 \pm 9.5	146	0.04	0.84
Number of fledglings	4.2 \pm 0.3	3.8 \pm 0.3	55	0.66	0.42
				U	P
Hatching success (%)	71.3 \pm 3.5	77.1 \pm 4.0		314	0.20
Mortality (%)	15.0 \pm 3.5	21.3 \pm 3.6		366	0.21
Fledging success (%)	60.0 \pm 4.0	57.7 \pm 3.5		431	0.78

were reduced because of brood parasitism by Great Spotted Cuckoos *Clamator glandarius* (Soler *et al.* 1998a, 1998b), depredation and desertion of nests (Table 1). Final sample size was 58 nests where at least one control and one food-supplemented nestling reached fledging, 30 nests in the arid habitat and 28 nests in the irrigated habitat.

Neck-collar trials

To study possible habitat differences in resource availability and/or nestling diets, we placed a neck-collar on 81 Great Spotted Cuckoo nestlings and 208 Magpie nestlings spread over 73 nests during the springs of 2002 and 2003 (46 and 27 nests, respectively), 36 in the arid habitat and 37 in the irrigated habitat. We used both Cuckoo and Magpie nestlings to increase sample size in assessing differences in resource availability between habitats; in a previous study no differences in diet were found between the two species (Soler *et al.* 1995). Neck-collars consisted of pieces (*c.* 80 mm) of fine wire (1 mm diameter) lined with plastic, placed around nestling necks when they were between 9 and 12 days old, allowing a normal respiration but avoiding the ingestion of food delivered by adult Magpies (Soler *et al.* 1995). After approximately 2.5 h, we went back to the nest to remove collars and collect food samples. There were no apparent effects of neck-collars, either on nestling health or on adult provisioning behaviour.

Food samples were stored in absolute alcohol. After identification, the samples were heat-dried in an oven at 60 °C for *c.* 24 h. The dry mass was then weighed to the nearest 0.0001 g. We obtained food

samples from 67% of the chicks. In most cases where we did not obtain food samples, chicks showed weight loss indicating that parents did not feed them during the time between placing and removing the collars. To calculate the mean dry food mass per nest, we only used chicks from which we obtained a food sample, and we took into account the time the collars remained on the chicks (mean dry food mass/h). The mean dry food mass was square root transformed to meet a normal distribution. Data were pooled across years. Although we have no information on the identity of individual birds, it was suspected, based on the proximity of nests, that only three territories could have been sampled in both years, so the influence of non-independent data points on the analysis is likely to be negligible.

To evaluate differences in diet between habitats, the following variables were calculated for each habitat: Shannon–Weaver's diversity index H' (Shannon & Weaver 1949); Sheldon's evenness e (Pielou 1975); and relative food item frequency $RX_i = X_i \times 100/X$ and relative food item abundance $RP_i = \Sigma RX_i/n$, where X is the total number of food items in each nest, X_i is the food item frequency and n is the number of nests.

Statistical analyses

Presence of ectoparasites in a nest was considered when at least one chick in the nest was infested (parasitized nests). We used stepwise generalized linear models (GLZ) and general linear models (GLM) to explore which of the measured variables best explained variation in, respectively, parasite prevalence

(present vs. absent) or intensity of infestation in the nest. As explanatory variables we considered habitat (two-level fixed effect) and the continuous variables clutch size, egg size, laying date, brood size and nest size. Differences in hatching success, nestling mortality and fledging success between habitats were analysed using Mann–Whitney *U*-tests.

We employed general linear mixed models (GLMM, PROC MIXED in SAS version 9.0; SAS Institute 2002) to assess simultaneously the effect of habitat and food treatment (two-level fixed factors), and their interaction, on nestling fitness traits (CMI, tarsus length, condition) and on the intensity of ectoparasite infestation. Laying date and brood size were introduced as covariates. Nestling condition was considered as nestling weight with tarsus length (structural size) introduced as a covariate (García-Berthou 2001, Freckleton 2002). When testing for CMI, nestling weight was used as a covariate. Nest identity (nested within habitat) and the interaction between food treatment and nest identity (nested within habitat) were random factors. The binary response variable presence/absence of parasites in nestlings was modelled with the GLIMMIX macro of SAS version 9.0 (Littell *et al.* 1996), using a logit link and a binomial error term. Degrees of freedom of the error terms of mixed models were estimated using the Satterthwaite method. Differences in nestling diet between habitats were analysed using multivariate analysis of variance (MANOVA), where the different relative food item frequencies in each nest were dependent variables, and habitat, year and its interaction the fixed effects.

RESULTS

Habitat and parental quality

Magpie pairs breeding in the irrigated habitat started to breed earlier, had somewhat smaller clutches, but showed no difference in egg size, nest size, hatching success, nestling mortality rate, fledging success or the number of fledglings compared with pairs breeding in the arid habitat (Table 1).

Habitat and nest infestation

The proportion of nests infested by *C. haemapterus* was exactly the same in irrigated and arid habitats (50%) and brood size was the only variable retained in the model to explain probability of nest infestation (GLZ $\chi^2_1 = 6.16$, $P = 0.013$, $n = 58$). The intensity of

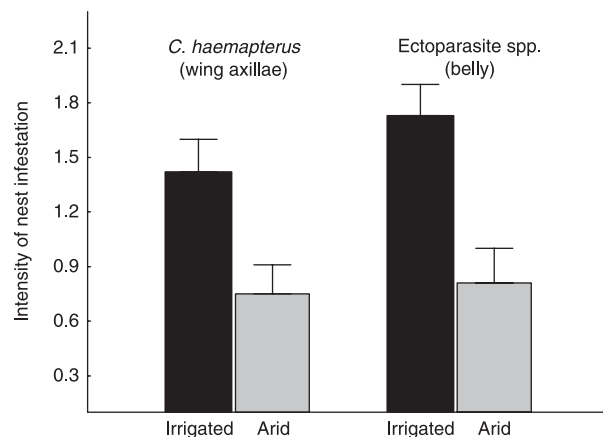


Figure 1. Results from GLM testing the effect of habitat on the intensity of nest infestation (spring 2001, $n = 29$ nests infested with *C. haemapterus* and 29 nests infested with ectoparasite spp.). The mean (\pm se) intensity of nest infestation on wing axillae and belly in irrigated and arid habitats is given. Nestling infestation ranges from 0 to 4.

nest infestation by *C. haemapterus* was significantly higher in the irrigated compared with the arid habitat (GLM $F_{1,27} = 7.57$, $P = 0.01$, $n = 29$, Fig. 1), while no other variables were retained in the model.

The probability of nest infestation by ectoparasite spp. and blood parasites depended primarily on the habitat (GLZ $\chi^2_1 = 9.10$, $P = 0.0025$ and $\chi^2_1 = 8.37$, $P = 0.0038$, respectively, $n = 58$). Thus, a significantly higher proportion of nests were infested in the irrigated (71.4 and 54% ecto- and blood parasites, respectively) compared with the arid habitat (30 and 10%). Additionally, the probability of nest infestation by blood parasites increased significantly as the season progressed ($\chi^2_1 = 7.34$, $P = 0.007$, $n = 58$). Other explanatory variables were not retained in the forward stepwise model (all $P > 0.15$).

The intensity of nest infestation by ectoparasite spp. was explained by the habitat (GLM $F_{1,27} = 7.98$, $P = 0.009$, $n = 29$, Fig. 1) and laying date ($F_{1,27} = 7.35$, $P = 0.012$). Other explanatory variables did not explain significant variation in the model (all $P > 0.45$). Moreover, in infested nests, 96% of nestlings showed traces of ectoparasite spp. in the irrigated habitat, while 57% did so in the arid habitat (GLMMIX $F_{1,26.5} = 5.64$, $P = 0.025$).

Habitat and nestling diet

After excluding food item groups with very rare occurrence (< 5%), we considered a total of 23 food

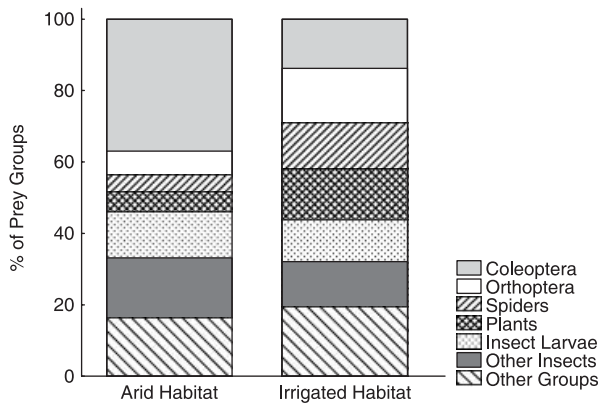


Figure 2. Composition of Magpie nestling diet in the arid and irrigated habitat (spring 2002–03, $n = 73$ nests).

item groups (S) in the Magpie nestling diet. The diversity index of food items was higher in the irrigated ($H' = 2.57$) compared with the arid habitat ($H' = 1.99$), which was also the case for the evenness index ($e = 0.57$ and 0.44 , respectively). This meant that the number of food items was more evenly distributed among food item groups in the irrigated compared with the arid habitat.

We reduced the number of food item groups to analyse differences in diet composition (relative abundances RP_i) between habitats: spiders, insect larvae, Orthoptera, Coleoptera, insects (containing all other insect orders), plants (almonds and cereals) and 'others' (containing all remaining food item groups). Considering these variables, diet composition did not differ between years, but did so between habitats (Year Wilks' Lambda = 0.87, $F_{7,63} = 1.38$, $P = 0.23$; Habitat Wilks' Lambda = 0.74, $F_{7,63} = 3.08$, $P = 0.0075$; Fig. 2). The interaction Year \times Habitat was not significant (Wilks' Lambda = 0.89; $F_{7,63} = 1.09$, $P = 0.38$). The groups that significantly differed between habitats in their contribution to diet composition were Coleoptera ($F_{1,69} = 12.33$, $P = 0.0079$) and Orthoptera ($F_{1,69} = 5.45$, $P = 0.022$), and approaching significance, spiders ($F_{1,69} = 3.33$, $P = 0.072$). Coleoptera constituted a relatively larger proportion of nestling diet in arid compared with irrigated habitat, while the opposite was the case for spiders and Orthoptera (Fig. 2). Mean dry food mass was significantly higher in 2002 than in 2003, but did not differ between habitats, nor was the interaction significant (Year $F_{1,68} = 6.31$, $P = 0.014$; Habitat $F_{1,68} = 0.17$, $P = 0.68$; Year \times Habitat $F_{1,68} = 0.19$, $P = 0.67$).

Table 2. Results of GLMM fixed effects testing the effect of habitat and food supplementation on Magpie nestling weight (condition), tarsus length, CMI and the intensity of *C. haemapterus* and ectoparasite spp. at the age of 17 days (spring 2001). Nest identity nested within habitat and the interaction between nest identity \times food were introduced as a random factor in all analyses. $n = 58$ nests (nestling fitness traits), $n = 28$ nests (*C. haemapterus*), $n = 29$ nests (ectoparasite spp.). Significant effects shown in bold.

	Explanatory term	df	F	P
Weight (condition)	Habitat	1, 58.2	0.35	0.56
	Food	1, 50.8	0.99	0.32
	Habitat \times Food	1, 50.9	1.98	0.16
	Laying date	1, 59.7	0.72	0.40
	Brood size	1, 59.1	12.69	0.0007
	Tarsus length	1, 206	294.09	< 0.0001
Tarsus length	Habitat	1, 54.7	8.87	0.0043
	Food	1, 49.4	0.15	0.70
	Habitat \times Food	1, 49.4	0.39	0.53
	Laying date	1, 57.4	5.43	0.023
	Brood size	1, 57.4	1.21	0.27
	CMI	Habitat	1, 55.4	9.40
Food		1, 46.9	25.29	< 0.0001
Habitat \times Food		1, 47	14.63	0.0004
Laying date		1, 63.1	2.11	0.15
Brood size		1, 64.4	0.29	0.59
Weight		1, 187	5.68	0.018
<i>C. haemapterus</i> (Axillae)	Habitat	1, 24.4	9.78	0.0045
	Food	1, 17.6	5.00	0.038
	Habitat \times Food	1, 17.7	1.66	0.21
	Laying date	1, 25.1	0.66	0.42
	Brood size	1, 26.6	0.11	0.74
	Ectoparasite spp. (Belly)	Habitat	1, 23.8	8.72
Food		1, 24	0.27	0.63
Habitat \times Food		1, 24	0.24	0.62
Laying date		1, 23.8	8.72	0.007
Brood size		1, 24.8	0.01	0.91

Habitat, food supplementation and nestlings

At 17 days of age, nestlings reared in the arid habitat showed significantly lower CMI but larger tarsus length compared with nestlings reared in the irrigated habitat (Table 2, Fig. 3). Nestling condition did not differ between habitats.

The food supplementation did not significantly affect tarsus length or nestling condition in either habitat type, but the effect of food supplementation on nestling CMI differed significantly between habitat types (GLMM Habitat \times Food $P < 0.001$, Table 2). The effect was not significant in nestlings reared in the irrigated habitat, whereas nestlings that received the food supplement in the arid habitat experienced a significant higher CMI compared with control nestlings (Fig. 3).

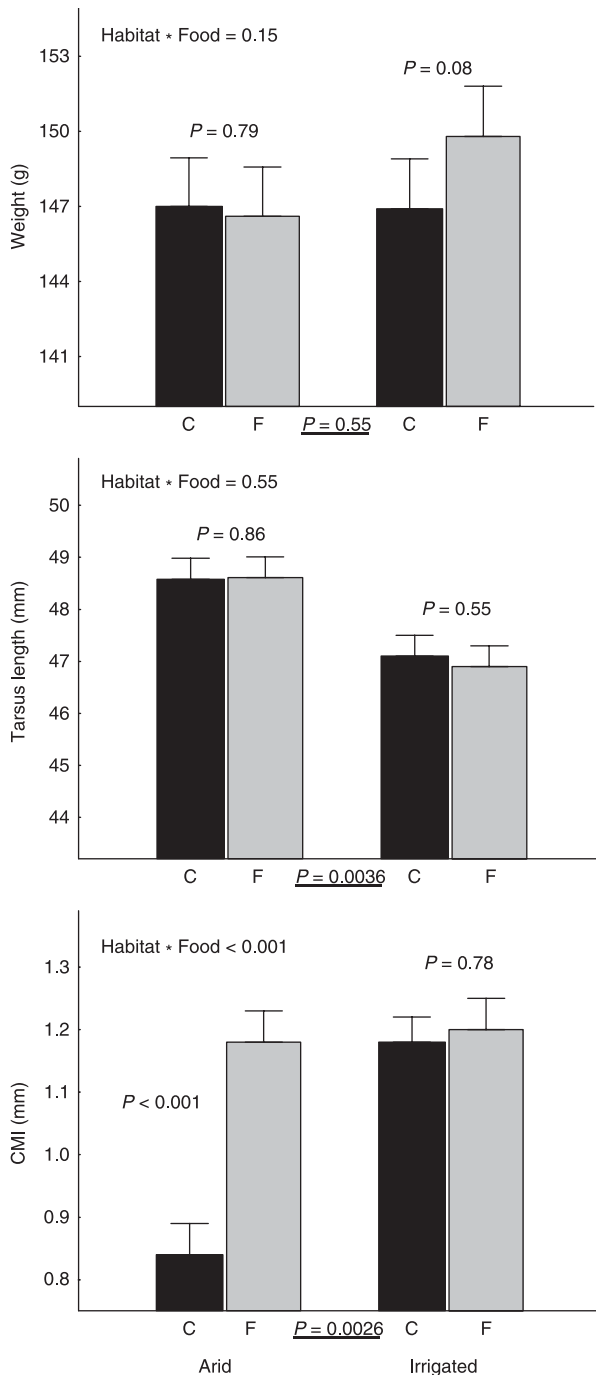


Figure 3. Weighted means (\pm se) of weight (condition), tarsus length, and CMI of control (C) and food-supplemented (F) nestlings in arid and irrigated habitats (spring 2001, $n = 58$ nests). P -values of GLMM are given for differences between habitats (underlined), for differences between food-supplemented and control nestlings in each habitat (*post-hoc* Scheffé test) and for the interaction between habitat and food supplementation.

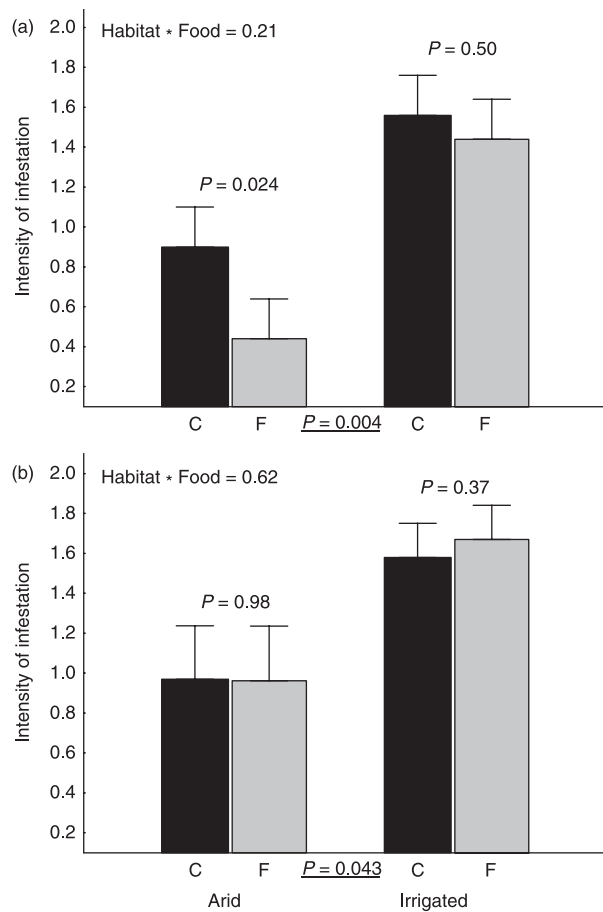


Figure 4. Weighted means (\pm se) of the intensity of infestation with (a) *C. haemapterus* ($n = 28$ nests) and (b) ectoparasite spp. ($n = 29$ nests) in control (C) and food-supplemented (F) nestlings in arid and irrigated habitats (spring 2001). P -values of GLMM are given for differences between habitats (underlined), for differences between food-supplemented and control nestlings in each habitat (*post-hoc* Scheffé test) and for the interaction between habitat and food supplementation.

Considering parasite-infested nests ($n = 29$ nests), food-supplemented nestlings showed significantly lower intensity of *C. haemapterus* (axillae) infestation compared with control nestlings (Table 2). However, *post-hoc* tests showed that this effect was only significant in the arid habitat (Fig. 4). Food supplementation did not affect the intensity of ectoparasite spp. (belly) infestation in either habitat (Table 2, Fig. 4).

The effect of food supplementation on the prevalence of blood parasites could only be analysed in the irrigated habitat, as only three nests were infested in the arid habitat. The prevalence of nestling blood parasites was not affected by food supplementation (GLMMIX $F_{1,22.3} = 0.99$, $P = 0.33$, $n = 15$ nests), but

showed a marginally positive relationship with brood size (GLMMIX $F_{1,22.6} = 3.86, P = 0.061$).

DISCUSSION

Habitat, parasite prevalence, nestling diet and parental quality

Magpie nestlings reared in the irrigated habitat suffered heavier infestations from both ectoparasites and blood parasites compared with nestlings reared in nests located in the arid habitat. This could be because vectors of several parasites may be more abundant in humid regions. For example, black flies (Diptera: Simuliidae) are vectors of several species of pathogenic avian leucocytozoans (Jamnaback 1973). These flies have aquatic larvae and pupae that require running water for their development (Crosskey 1990). In accordance with previous findings on habitat-related parasite prevalence (Van Riper 1991, Moyer *et al.* 2002, Galeotti & Sacchi 2003), our results suggest that the irrigated habitat has a higher parasite pressure compared with the arid habitat. Thereby, it is possible that breeding Magpies could use territory characteristics to evaluate the future risk of parasitism for their offspring.

Our results suggest that the diet in the arid habitat was less varied than that in the irrigated habitat and diet composition differed between habitats in relation to Coleoptera, Orthoptera and Araneae, three of the most important diet components of Magpie nestlings. In addition, Magpie breeding density was higher in the irrigated than in the arid habitat (Soler *et al.* 1998), but reproductive success (fledging success and the number of fledglings), nest size, egg size and also the amount of food received by nestlings did not differ between habitats. These results could thus indicate that Magpies in the arid habitat breed in larger territories compared with those breeding in irrigated areas, allowing similar resource availability in both habitats. However, Magpie pairs breeding in the irrigated habitat did start breeding earlier and laid rather smaller clutches compared with those in the arid habitat. Smaller clutches did not lead to a significantly lower number of fledglings, even though fledgling success was very similar in both habitats. This may be due to the difference in sample size for the two measurements (Table 1). Varying clutch size and laying date have previously been suggested as possible effective strategies to reduce the effect of parasites, because individuals that start to breed earlier and lay smaller clutches in a high pathogenic

environment have shown benefits (Blondel 1985, Richner & Heeb 1995, Møller 1997, Saino *et al.* 2002b, Fargallo & Merino 2004). However, it was not possible to disentangle the interaction between habitat pathogenesis and clutch size/laying date on reproductive success in the present study.

Habitat, food supplementation and nestling quality

Fledglings reared in the arid habitat had significantly larger tarsus length and a lower CMI compared with fledglings reared in the irrigated habitat, but they did not differ in body condition. Therefore, it seems that chicks raised in the arid habitat invested less in immune defences, attaining a larger body size. These differences in nestling fitness traits between irrigated and arid habitats could be attributed to several factors, but two of the most important factors are likely to be nutrition and parasite selection pressure (Lochmiller & Deerenberg 2000).

A difference in resource availability between habitats may result in different provisioning rates of adults breeding in these habitats, but results of the neck-collar trials do not support this possibility. According to the parental food compensation hypothesis, parents might have increased feeding effort in response to higher parasite pressure (Christe *et al.* 1996, Tripet & Richner 1997, Hurtrez-Boussès *et al.* 1998, Bouslama *et al.* 2002) and nestlings in the irrigated habitat could therefore have reached higher CMI. One possible proximate mechanism for this parental response may be an increase in begging calls of parasitized chicks (Christe *et al.* 1996). However, we found that mean dry food mass was not higher in the irrigated compared with the arid habitat, suggesting a similar ingestion of the amount of food between habitats. Another possibility is that the higher CMI in irrigated habitats and the higher growth in arid habitats was a consequence of differences in nestling diet between habitats. Nestling diet in the irrigated habitat tended to be more varied, and diet composition was significantly different between habitats. This could indicate a different amount of micronutrients and amino acids necessary for optimal immune development between habitats. Unfortunately, we have no information with respect to specific prey species enhancing the development of the immune system or growth.

The food supplementation experiment had a different effect on nestlings reared in the arid habitat compared with nestlings reared in the irrigated

habitat, especially when looking at the effect on nestling CMI. The fact that the food supplement only significantly affected CMI, but not growth (tarsus length) *per se* is possibly a consequence of the nature of the food supplement which, apart from being calorie-rich, also contained essential micronutrients and vitamins that enhance the development of the immune system (Friedman & Sklan 1997, Klasing 1998, but see De Neve *et al.* 2004 for a further explanation). In addition, the small amount (0.1 mL) provided every 2 days was perhaps not sufficient to affect growth significantly. The effect of food supplementation on CMI was much stronger in nestlings reared in the arid habitat, while non-significant in nestlings reared in the irrigated habitat (Fig. 3). This interesting difference between habitats in the effect of food supplementation on CMI does support the hypothesis of different priority rules for the allocation of resources to immune system development in association with the pathogenicity of the rearing environment (Lochmiller & Deerenberg 2000). The pathogenicity of the environment could be a triggering factor for differential investment in immune defence. If in a higher pathogenic rearing environment all nestlings (food-supplemented or control) allocated resources primarily to the development of the immune system, food supplementation would not help to increase immune defence, because immune defence is already at its optimum (see similar maximum levels of CMI in the irrigated and food-supplemented nestlings of the arid habitat; Fig. 3). Food supplementation would not influence the probability of being infested in this case. Indeed, food-supplemented and control nestlings in the irrigated habitat showed similar CMI and were equally infested by parasites (Fig. 4). Nestlings in low pathogenic environments (arid habitat) probably invested resources primarily in growth. Here, the food supplementation increased CMI, but had no effect on condition or tarsus length (Fig. 3). Interestingly, an enhanced immune function of food-supplemented nestlings in the arid habitat apparently allowed better defence against ectoparasite attacks, because food-supplemented nestlings experienced lower infection load of *C. haemapterus*.

One possible mechanism of different priority roles for the allocation of resources to two competing activities such as growth and immune development may involve differential maternal investment in eggs. Depending on the risk of parasitism, mothers could favour differential investment in growth or immunity of their offspring by differential transmission of maternal antibodies and allocation of antioxidants (Buechler

et al. 2002, Saino *et al.* 2002a, 2003, Tschirren *et al.* 2004). These mechanisms, however, may represent a significant cost for the female, and should therefore be adjusted relative to the risk of infection in the offspring.

In conclusion, nestlings invested differentially in growth or immunocompetence depending on the breeding habitat, suggesting limited resource availability to fuel both physiological processes optimally. In addition, the effect on immunocompetence of food supplementation to nestlings differed between habitats. Differences in parasite pressure and nestling diet between habitats may have driven the different strategies towards an increased investment in immunocompetence or growth. A simultaneous manipulation of food and parasites would be necessary to disentangle the effects of both factors on nestling immunocompetence.

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