Effects of food abundance and predictability on body condition and health parameters: experimental tests with the Hooded Crow

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It has been shown that small passerines can counteract variability of food resources by actively regulating their body reserves through an increase of mass. However, the effects of food predictability on body mass regulation and other body parameters, such as immune functions, in larger species have been little studied. To analyse the response of the Hooded Crow *Corvus corone* to food abundance and predictability, we performed three experiments with controlled food provisioning under laboratory conditions. Body mass, TOBEC (total body electrical conductivity) lean mass index, blood parameters and immune organ masses were measured at the beginning and end of a 15-day period. In the first experiment, the food release was predictable (same amount each day) but the quantity of food delivered to five groups of birds varied (37, 75, 100, 150 or 300 g/day). Low food levels induced a greater decrease in mass accompanied by an increase in erythrocyte sedimentation rate. In the second experiment, the same average quantity of food (100 g) was supplied according to either predictable or unpredictable (random) schedules. In this case, the crows lost more mass, and their erythrocyte sedimentation rate increased when food was unpredictable. In the third experiment, the same average quantity of food (150 g) was supplied according to either a predictable schedule or two schedules with different levels of variability. The group with a low level of variability did not differ from the control, while the group with a highly variable feeding schedule lost more mass. In this group, the higher mass loss was associated with greater variation of the erythrocyte sedimentation rate and a reduced Phytohaemag-glutinin index of immunocompetence and haematocrit value. The results of experiments 2 and 3 contrast with findings in other passerines, which increase their mass when food availability is unpredictable. It appears that a body mass decrease in the Hooded Crow can be induced by a reduction of either food abundance (exp. 1) or food predictability (exp. 2, 3), and it is accompanied by a worsening of health state.

A knowledge of the dynamics of energy reserve accumulation and mobilization is important in assessing the costs and benefits of many ecological adaptations, both in the reproductive season and in other periods (migration, wintering). Studies of some birds indicate that they actively regulate their body reserves, e.g. storing higher quantities of fat, when forced to confront periods when food resources are scarce (Stuebe & Ketterson 1982, Gosler *et al.* 1995; Gosler 1996) or unpredictable (Ekman & Hake 1990, Rogers & Smith 1993, Bednekoff & Krebs 1995, Witter *et al.* 1995, Cuthill *et al.* 2000). Recent studies have placed the energy budget within a more general picture of trade-offs between different body compartments (Norris & Evans 2000). In particular, since animals must use resources for maintenance of the immune system, it can be hypothesized that a shortage of food can cause a worsening of health conditions (Klasing 1988, Gustafsson *et al.* 1994). The immune system plays a pivotal role in defending an animal against attack by pathogens and parasites (Roitt *et al.* 1998). In theory, there are trade-offs between investments in life-history components and immune defence (Norris & Evans 2000).

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Most experiments on nutritional stress have focused on the effects of food quantity on haematological and immunological parameters, while studies considering the effect of food variability are rarer.

A prolonged shortage of food has various effects on birds. In addition to an obvious weight loss, fasting may cause an alteration of the haematocrit value (Ferrer *et al.* 1987, Romero-Pujante *et al.* 1999) or an increase in the heterophil/lymphocyte ratio (Romero-Pujante *et al.* 1999, Totzke *et al.* 1999). Tissue catabolism of proteins is always altered after fasting (Garcia-Rodriguez *et al.* 1987). Moreover, the protein composition of the diet could influence the development of organs associated with immuno-competence (Lochmiller *et al.* 1993).

It is difficult to compare the results of studies assessing the effects of nutrition on different immune system components. This is due to the different fasting schedules used, and because it is possible that the limitation of resources has a different effect on the various components of the immune system. This could occur if individuals invest strategically in different components of their immune system (Norris & Evans 2000).

In contrast to food scarcity, food predictability has prompted great interest in evaluating the effects on the body's energy reserves (weight, fat accumulation) but not on health state. The response to food predictability falls within a risk-sensitive optimal foraging approach (Krebs & Davies 1993) and has been studied mainly in small passerines. These animals are able to regulate their internal (Bednekoff & Krebs 1995) and external (Hurly 1992) energy reserves in response to or in anticipation of requirements. To avoid the risk of starvation when resources become unpredictable, there is an increase in food caches or in weight. However, this fattening strategy is not universal, since a different response was recently described in Japanese Quail *Coturnix coturnix* chicks: individuals subjected to unpredictable nutrition lose weight with respect to controls fed regularly (Boon *et al.* 1999). This indicates that they respond only to their energy needs at the moment, and seem not to anticipate the food restriction period nor to respond to feeding conditions experienced on previous days. The fact that individuals subjected to food unpredictability exhibit poorer body condition suggests that they are also less immunocompetent. However, there have been no studies of the effect of predictability on health state.

The aim of our study was to assess the responses of the Hooded Crow *Corvus corone* to food shortage and food variability, in terms of both accumulation of reserves and immune response. We utilized a multitechnique approach to assess body and health conditions in 99 Hooded Crows subjected to three experimental protocols:

1 to evaluate the effects of food quantity, we fed Hooded Crows with a daily quantity of food that was constant but different according to the group (37 g, 75 g, 100 g, 150 g, 300 g). The different quantities of food delivered were chosen to provide a range of food abundance encompassing the predicted basal metabolic rate (289.9 kJ, i.e. 52.1 g, Aschoff & Pohl 1970) and the values reported by Jollet (1984: 61–83 g).

2 and 3 to determine the effects of food variability, we compared groups given a constant quantity of food (100 g or 150 g) with groups in which the quantity of food varied from day to day (although the daily averages were the same as in the controls).

METHODS

The study was carried out at Rivalta Scrivia (44°51′N, 08°48′E; NW Italy) in the spring of 1998 and 1999. Ninety-nine Hooded Crows were captured with Larsen traps as part of the provincial administration's agricultural pest management programme. No birds were trapped exclusively to collect scientific data. Measurements were taken on two occasions. At the beginning and at the end (15 days later) of the experimental treatment, we measured (*in vivo*) each individual's mass and lean mass index with a TOBEC (total body electrical conductivity) apparatus, while blood samples and smears were collected for subsequent analyses. After the experimental treatment, the birds were killed by cervical dislocation (by personnel from the provincial administration authorized to cull crows by the National Wildlife Institute), whereupon each bird's body was brought to the laboratory, where morphological variables and internal organs were measured.

Morphological variables, internal organs and body composition

We measured: (i) body mass (0.1 g accuracy); (ii) wing length, with the flattened wing outstretched perpendicular to the body; (iii) tail length; (iv) tarsus length; (v) bill length. Tarsus and bill measurements were made to the nearest 0.1 mm with calliper, while all other variables were measured to the nearest 0.5 mm with a flat ruler. The four body measures (ii to v) were highly correlated with each other; thus

they were summarized in an index of structural size calculated as the first Principal Component from a PCA. Component 1 explained 59.3% of the variance in the original variables and was highly correlated with them (loadings: wing = 0.814, tail = 0.793, tarsus = 0.737, bill = 0.733).

We examined the abdominal cavity of dead birds to extract internal organs. We weighed the spleen and bursa of Fabricius on a precision balance with 0.1 mg accuracy.

In live birds, the body composition was evaluated non-invasively by measurement of electroconductivity with the TOBEC system. This device estimates lean body mass, since the contribution of lipid tissue to conductivity is negligible (Walsberg 1988, Castro *et al*. 1990). We placed each individual in the detection chamber (152 mm diameter) of an SA-3000 Small Animal Body Composition Analyser (EM-SCAN Inc., Springfield, IL, USA) for 5–6 s and recorded the electrical conductivity index: TOBEC index = $(S - E)/R$, where S = measurement with the specimen, E = empty measurement, and R = reference number (detailed instructions in the EM-SCAN operator's manual). For each individual the measurement was repeated three times and the average value used for statistical analyses.

Leukocyte counts, erythrocyte sedimentation (ES) rate and haematocrit

We counted leucocytes and red blood cells in blood smears stained by the May–Grunwald–Geimsa method. Blood smears were scanned at 630× magnification following standard routines. In each microscopic field, we counted red blood cells and leucocytes classified as lymphocytes, monocytes, eosinophils, heterophils and basophils. In each smear, we counted 100 leucocytes and the corresponding red blood cells. We then calculated the relative frequency of leucocytes of each family of leucocytes with respect to the total leucocyte population (relative counts) and the number of leucocytes of each family per 10 000 red blood cells (absolute counts). This method provides significantly repeatable relative and absolute leucocyte counts (Saino *et al*. 1995). Absolute values were log10 transformed for statistical analysis.

To measure the sedimentation rate, 70-µL blood samples in heparinized haematocrit capillary tubes were placed in a vertical position for 4 h in a refrigerated container (4 °C). According to Saino and Møller (1996), the ES rate, i.e. the proportion of blood forming a sediment per hour, was expressed as: (volume of the part of the capillary not occupied by blood cells)/(blood volume in the capillary) × 0.25. In this formula the ×0.25 multiplication is required to obtain an hourly value after 4 h of sedimentation. In the 'high variability 150 g' group (the description of groups is reported below), blood samples were then centrifuged for 10 min at 4000 r.p.m. Haematocrit was expressed as volume of the part of the capillary occupied by blood cells/blood volume in the capillary.

Phytohaemagglutinin index

In the 'high variability 150 g' group, the thickness of the right wing web (patagium) was measured with a spessimeter with an accuracy of 0.01 mm at the end of the experimental period. The wing web was then injected with 0.25 mg of phytohaemagglutinin (PHA: Sigma L-8754) in 0.05 mL of phosphate-buffered saline (PBS). Twenty-four hours later, we again measured the thickness of the wing web at the inoculation site. Reaction to PHA was expressed as the change in thickness of the inoculated wing web. This wing web index is routinely applied in avian studies and is assumed to be proportional to the intensity of T-lymphocyte cell-mediated immunocompetence (Lochmiller *et al*. 1993, Smits *et al*. 1999).

Experimental protocol

After capture, all birds were kept in individual cages (100 × 60 × 80 cm) for 15 days and received different diets. All birds had water *ad libitum* and were cared for in accordance with the principles of the Association for the Study of Animal Behaviour. As food we used a mixture of meat (33%), fruit (53%) and maize (14%). For each individual, we calculated the daily energy intake using a standard table of conversion from g to kJ (Italian Nutritional Institute: meat = 8.96 kJ/g; fruit = 1.42 kJ/g; maize = 14.86 kJ/g). Food was dispensed individually in poultry seed cups at 09:00 h. We measured the food consumption daily and corrected for food spillage by weighing the spilled food.

We assigned the crows to different treatment groups using a completely randomized procedure. We established three experimental protocols:

Experiment 1: to evaluate the effects of food quantity, we fed the birds with daily quantities of food that were constant but different in the five groups: 37 g (seven individuals (ind.)), 75 g (eight

ind.), 100 g (14 ind.), 150 g (16 ind.) and 300 g (seven ind.).

Experiment 2: to evaluate the effects of food variability, we established one group with a variable schedule. Each bird was provided with a different quantity of food each day. The daily amount of food was determined by extraction of random numbers, and individuals pertaining to this group had different schedules but an identical mean and sd (100 ± 49 g/day) at the end of the study period. The group comprised 17 individuals. As a control, we used the 14 birds from the 100 g constant group of experiment 1.

Experiment 3: to evaluate the effects of food variability, we established two groups with different schedules. Each bird was provided with a different quantity of food each day, and individuals pertaining to each group had different schedules but an identical mean (150 g/day) and sd at the end of the study period. The groups were:

1 low variability (seven ind.): the 15-day average was 150 g/day, with a ±35 g sd;

2 high variability (26 individuals): the 15-day average was 150 g/day, with a ±74 g sd.

As a control, we used the 16 individuals from the 150 g constant group of experiment 1.

RESULTS

Experiment 1: food quantity effects

The five experimental groups were similar for all the variables at the beginning of the experimental period (Table 1). At the end of the period of differential nutrition, the groups differed in mass, TOBEC lean mass index and ES rate variation (Table 1). Leukocyte variation and immunocompetent organ mass did not differ between treatment groups. The mass loss was considerable for birds of the 37 g group, subjected to a harsh diet, and it was moderate for birds of the 75 and 100 g groups. In contrast, birds of the 150 and 300 g groups, which had abundant food, gained mass slightly (Fig. 1). The ES rate was much higher at the end of the experiment than at the beginning in the 37 g group, whereas the other groups had ES rate variations close to zero (Fig. 2).

Experiment 2: food variability effects

Mass and ES rate variations differed significantly between the 100 g variable group and its 100 g control (Table 2). Leukocyte variation and

Table 1. Experiment 1: comparison of mean values of mass, body size, internal organs, and haematological parameters in five groups fed with different quantities.

Parameter	Difference among five groups	
	F	*P*
Beginning of experimental period		
Mass	0.67	0.62
Size (PCA factor 1)	2.40	0.06
Lean mass index	1.55	0.23
Fat index	1.21	0.32
ES rate	0.29	0.88
Leukocytes[1]	2.27	0.08
Heterophils[1]	1.84	0.14
Lymphocytes[1]	1.51	0.22
Heterophils (%)	0.64	0.64
Lymphocytes (%)	0.41	0.80
H/L ratio	0.53	0.72
End of experimental period		
Mass variation	32.6	0.001***
Lean mass index variation	4.1	0.015*
Fat index variation	2.22	0.11
Spleen	1.69	0.17
Bursa	1.78	0.15
ES rate variation	6.32	0.001***
Leukocyte variation	2.39	0.07
Heterophil variation	1.03	0.41
Lymphocyte variation	2.38	0.07
H/L ratio variation	0.51	0.73

[1]Leukocytes, heterophils, and lymphocytes abundances calculated as N/10 000 red blood cells.
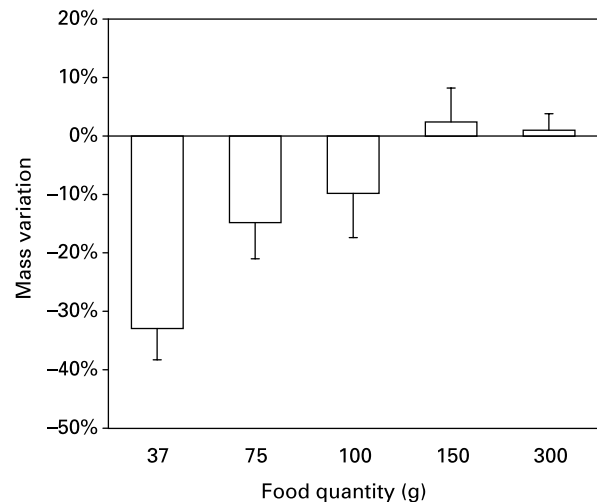

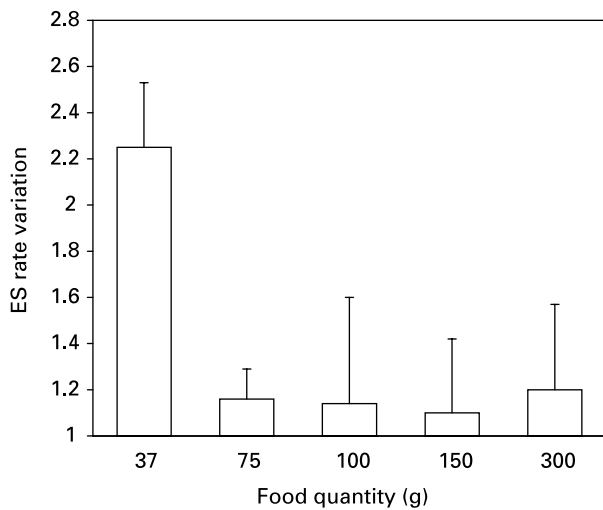**Figure 1.** Comparison of body mass variation in five groups fed with different quantities.

Figure 2. Comparison of ES rate variation in five groups fed with different quantities.

Table 2. Experiment 2: comparisons of mean values of mass, body size, internal organs, and haematological parameters in two groups fed with a 100 g/day mean and different schedules (control and variable).

Parameter	Difference between groups	
	F	P
Beginning of experimental period		
Mass	0.63	0.43
Size (PCA factor 1)	2.19	0.15
Lean mass index	0.02	0.88
Fat index	0.01	0.98
ES rate	1.01	0.32
Leukocytes	1.76	0.20
Heterophils	1.59	0.22
Lymphocytes	2.10	0.16
Heterophils (%)	0.34	0.57
Lymphocytes (%)	0.49	0.49
H/L ratio	0.96	0.34
End of experimental period		
Mass variation	4.58	0.041*
Lean mass index variation	2.63	0.14
Fat index variation	2.18	0.18
Spleen	1.56	0.22
Bursa	0.70	0.41
ES rate variation	4.18	0.05*
Leukocyte variation	2.47	0.12
Heterophil variation	2.95	0.10
Lymphocyte variation	1.60	0.22
H/L ratio variation	0.08	0.93

immunocompetent organ masses did not differ between treatment groups. The birds of both groups lost mass during the experimental period, but those of the 100 g variable group had greater losses (Fig. 3).
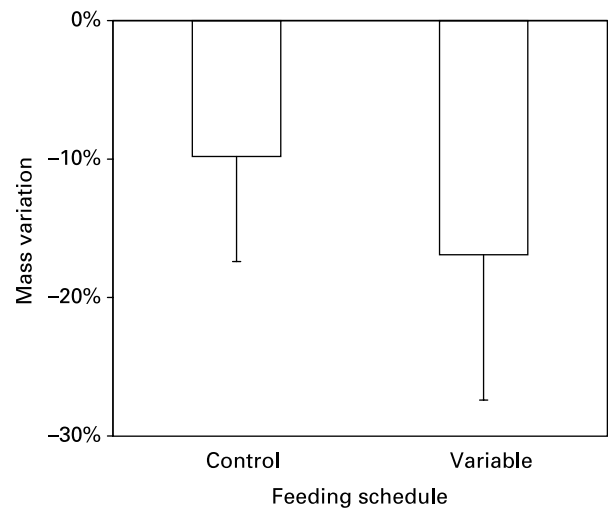

Figure 3. Comparision of body mass variation in two groups fed with a 100 g/day mean and different schedules (constant and variable).

The ES rate variation was significantly higher in the group nourished with the variable schedule.

Experiment 3: food variability effects

In this experiment, the control was the 150 g group (these birds were given 150 g of food daily) for both the groups with variable schedules. There were no significant differences in the variables between the 150 g low variability group and the control (Table 3).

Only the mass and the lean mass index variations differed significantly between the 150 g high variability group and the control (Table 3). The control birds increased slightly in mass during the experimental treatment, whereas the birds with a high variability schedule lost mass (Fig. 4). Birds of the latter group did not consume all the food available when the quantity of daily food provided was greater than the amount they could ingest (typically no more than 200–220 g/day).

In the high variability group, we measured both energy intake of ingested food and ES rate in 17 individuals, while PHA injection and haematocrit assessment were done on the other nine individuals. The mass loss was correlated (Fig. 5) with energy intake during the 15 days ($r_{17} = 0.675$, $P < 0.01$) and ES rate variation ($r_{17} = -0.501$, $P < 0.03$). In the other nine individuals tested, the mass loss was significantly related to PHA index ($r_9 = 0.778$, $P < 0.01$) and haematocrit value ($r_9 = -0.553$, $P < 0.04$).

Table 3. Experiment 3: comparisons of mean values of mass, body size, internal organs, and haematological parameters in three groups fed with a 150 g/day mean and different schedules (control, low variability, and high variability).

Parameter	Control vs. low variability		Control vs. high variability	
	F	P	F	P
Beginning of experimental period				
Mass	1.14	0.30	1.10	0.30
Size (PCA factor 1)	0.26	0.62	0.42	0.52
Lean mass index	1.08	0.31	1.54	0.24
Fat index	3.60	0.07	0.27	0.61
ES rate	0.01	0.94	0.03	0.86
Leukocytes	2.95	0.10	0.69	0.41
Heterophils	0.84	0.37	2.38	0.14
Lymphocytes	3.27	0.09	0.50	0.48
Heterophils (%)	1.37	0.26	0.01	0.93
Lymphocytes (%)	1.70	0.21	0.01	0.97
H/L ratio	1.62	0.22	0.02	0.88
End of experimental period				
Mass variation	0.16	0.69	15.8	0.001***
Lean mass index variation	0.35	0.56	4.53	0.04*
Fat index variation	0.68	0.42	1.36	0.25
Spleen	0.85	0.37	2.21	0.15
Bursa	1.81	0.19	0.01	0.98
ES rate variation	0.12	0.74	0.02	0.89
Leukocyte variation	1.04	0.32	0.10	0.76
Heterophil variation	1.92	0.18	0.01	0.94
Lymphocyte variation	3.61	0.07	0.13	0.72
H/L ratio variation	2.02	0.17	0.80	0.38

DISCUSSION

Food abundance

In the Hooded Crows, the differences in food abundance led to strong variations in mass, a parameter that has been shown to be critical for reproduction (Loman 1984). Mass is probably a very sensitive parameter; in a parallel study of free-living individuals, in which we analysed variations of the same set of parameters, we found year-to-year fluctuations in mass while no other body or health condition indices varied (Acquarone *et al.* 2001a, 2001b). In our experimental situation, the lower food supply (37 g/day for 15 days) caused a mass decrease that was mainly due to lean mass consumption, as detected by the TOBEC technique. The contribution of body fat reserve was negligible; indeed, in this species the percentage of body lipids is less than 3% (Acquarone *et al.* 2001b). The other four groups, which received a higher amount of food (from 75 to 300 g), roughly maintained the initial body mass. This result is in
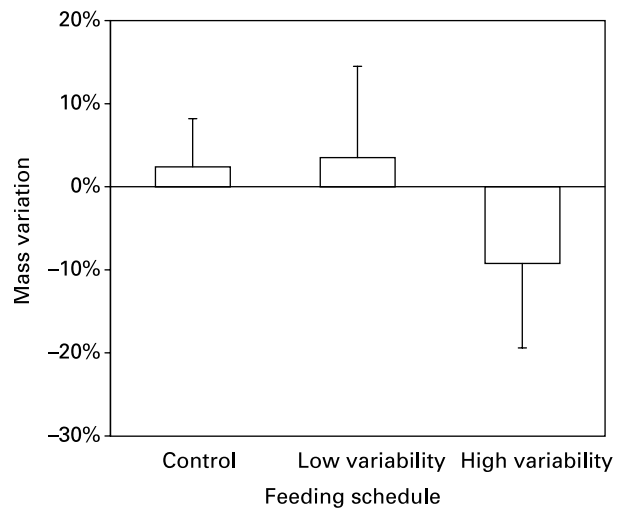

Figure 4. Comparison of body mass variation in three groups fed with a 150 g/day mean and different schedules (constant, low variability, and high variability).

accord with Jollet (1984), who reported 61–83 g as the estimated daily intake for this species. It should be noted that birds fed with the largest amounts did not increase their mass proportionally because the individuals with extra food did not eat all of it. There are several possible adaptive hypotheses to explain why individuals with extra food did not respond by gaining mass; some of these will be discussed in the next section in the light of results on food predictability.

The daily food shortage also caused a change in one immunological parameter, i.e. an increased ES rate, while absolute and relative counts of white blood cells did not vary. In fasting experiments, alterations of metabolites are usually reported (Garcia-Rodriguez *et al.* 1987, Ferrer & Dobado-Berrios 1998), while variations in immunological parameters have less often been studied. Among the set of parameters or tests that can be employed, some have been shown to vary in response to fasting, i.e. haematocrit, lymphocytes/heterophils ratio, the PHA and SRBC tests (Romero-Pujante *et al.* 1999, Totzke *et al.* 1999). As Norris and Evans (2000) pointed out, the use of a single standard reliable indicator of health in birds is still being debated. Because different parts of the immune system can be affected differentially by nutrition and external stress factors, if individuals invest strategically in different components of their immune system, there will not be a correlated response in the measured health parameters (Norris & Evans 2000).
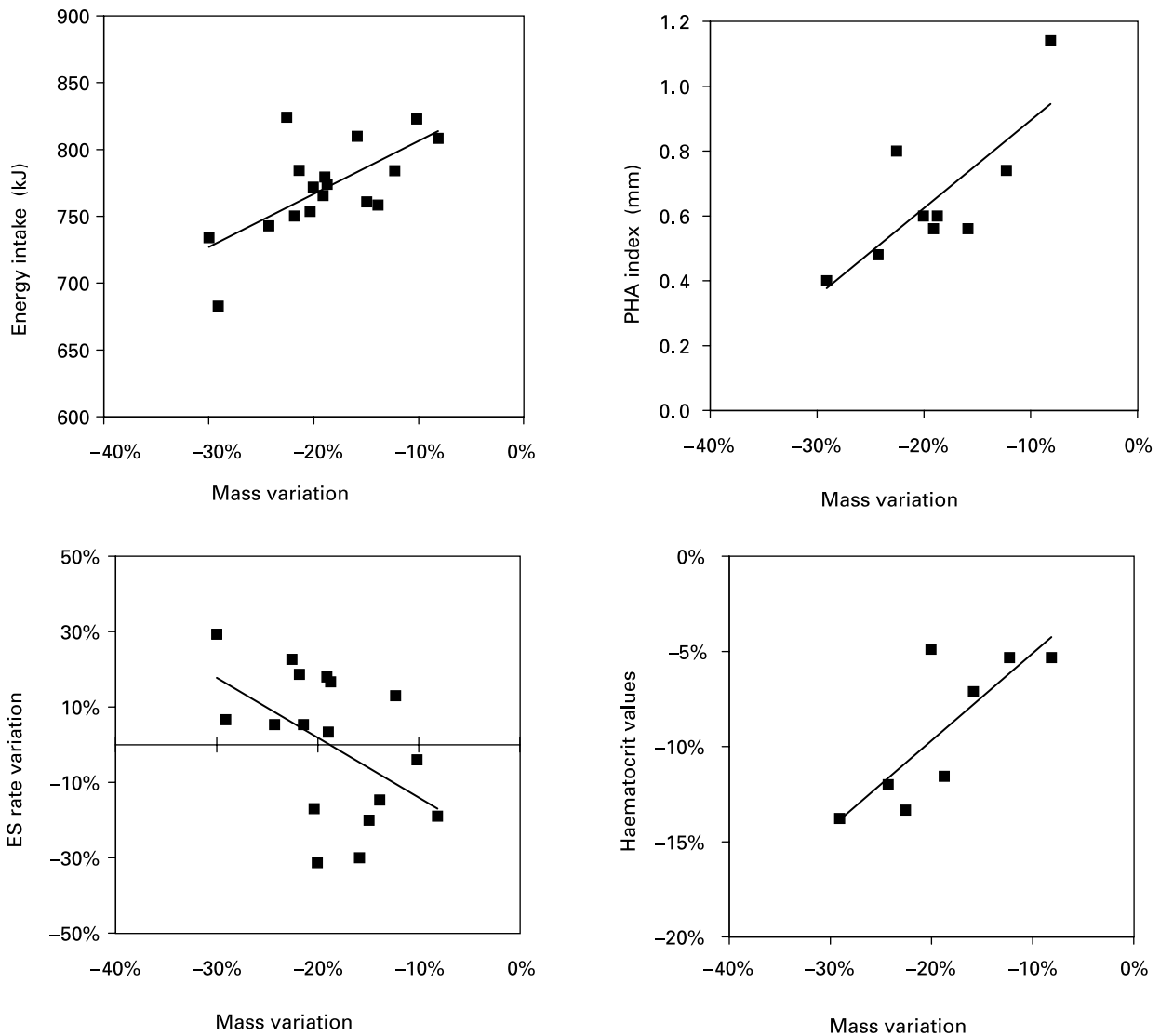
Figure 5. Relationship between mass variation and energy intake during the 15-day period, ES rate variation, PHA index and haematocrit values (150 g/day high variability group).

In the Hooded Crow, the ES rate appears to be a more sensitive parameter, or more related to body mass condition, than absolute and relative counts of blood cells. We have reached the same conclusion by monitoring the same set of parameters in free-living individuals, as we observed that the ES rate was the only health state parameter related to mass values, lighter individuals having a higher ES rate (Acquarone *et al.* 2001b).

Food predictability

Hooded Crows responded to daily predictability of food supply by decreasing their mass more than controls in both experiments 2 and 3. This pattern is similar to the one reported for Japanese Quail chicks (Boon *et al.* 1999). Indeed, neither study fitted the classical model of fattening (adaptive response hypothesis) in unpredictable situations (Lima 1986). Our results for the Hooded Crow can be discussed in the context of the following points: (1) mass variation can be affected by two proximate causes, namely (a) the existence of a limit in the capacity to ingest food, and (b) the sort of body energy reserves mobilized in the food-shortage periods. The existence of a limit in the capacity to ingest food could have played a role only in the third experiment. Here the individuals of the high

variability group did not consume all the food available when the quantity of daily food provided was greater than the amount they could ingest in a day (about 200–220 g). Thus, on average these birds consumed less food during the 15-day period of the experiment. However, it is likely that the ingestion limit only had an additional effect on the bird's mass decrease. Indeed, in experiment 3 the mean mass decrease of the variable group was about 10% more than the control group, while their energy intake was only reduced by about 6% because of the ingestion limit. Moreover, a decrease in mass also occurred when Hooded Crows ate all the food, as in the variable group of the second experiment. With regard to (b), an unpredictable food supply can in some way result in more energy being used on average by a bird. The mechanism for this could be, for example, that birds with less food need constantly to mobilize inefficient energy stores like muscles (Blem 1990) on days when little food is received, and then have to rebuild them on days when more food is available. In our study species, without a substantial lipid storage, the mobilization of other substances is probably the rule, but further physiological studies are needed to clarify this aspect. Some ultimate reasons for lack of fattening in the Hooded Crow will be considered in points 3–5 below. (2) Our study was conducted in spring, when climatic conditions are less extreme than in winter. While describing the adaptive response hypothesis under periodic food availability in the Starling *Sturnus vulgaris*, Witter *et al.* (1995) discussed the limits to a generalization of the model, since mass regulation at different times of the annual cycle changes considerably. This may be related to season-specific changes in environmental stochasticity. It can be supposed that only an interaction of food predictability with harsher conditions (e.g. in winter) would cause the appearance of internal or external adaptive responses. (3) Fattening strategies might be more useful in small birds because of their relatively high metabolic requirements and high risk of starvation (Blem 1990), while larger birds like crows have a greater margin for survival in environmental extremes. (4) Crows are opportunistic resident birds that live in agricultural ecosystems, which are generally food-rich habitats. Human-generated resources are probably more predictable than natural ones. (5) As an alternative to energy storage, Hooded Crows can occasionally hoard food and use these reserves when food is not available (Fjeld & Sonerud 1988); thus in this species, the costs of fattening (Lima 1986, McNamara & Houston 1990) could outweigh the benefits.

Considering our results and those of others (Boon *et al.* 1999), it seems that there is a need for more data before a detailed theory of mass variation or, more widely, of energy allocation in birds under unpredictable situations can be formulated.

In this paper, the effects of food predictability on health state have been considered. In experiment 2 (100 g/day), the group subjected to an unpredictable feeding schedule showed a higher ES rate than controls, suggesting that mass loss is accompanied by a negative effect on health condition. In experiment 3 (150 g/day), there was no difference in ES rate between the high variability group and the controls. However, in this case the lack of difference could be due to a threshold effect: even if each individual experienced days with food shortage, the birds never reached the same poor condition as those in experiment 2. In view of the negative effects of mass loss on ES rate, the PHA test of immunocompetence and the haematocrit values in the birds of experiment 3, we can infer that individuals subjected to an unpredictable schedule and showing a greater mass loss also suffered negative effects on their health condition.

In conclusion, it appears that a body mass decrease in the Hooded Crow can be induced by a reduction of either food abundance or food predictability, and it is accompanied by a worsening of health state.

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