

NOTE:

**BODY CONDITION, IMMUNOCOMPETENT ORGANS, AND BLOOD  
PARAMETERS IN THE HOODED CROW**

CAMILLA ACQUARONE, MARCO CUCCO,\* AND GIORGIO MALACARNE

*Department of Sciences and Advanced Technology, University of Eastern Piedmont,  
via Cavour 84, I-15100 Alessandria, Italy*

In ecological studies, it is acknowledged that body reserves and health condition can affect Darwinian fitness (Pravosudov and Grubb, 1997). Since body mass alone does not usually reflect body condition (Blem, 1990), precise measurements of nutritional and health parameters are necessary to estimate the ability of animals to confront specific environmental demands. Recently, in addition to traditional morphological indicators of fat accumulation (mass, fat score, abdominal profiles), body reserves have been measured by electrical conductivity (Scott et al., 1991). Another recent development in ornithological studies has been the application of immunological tests with both cellular and plasma hematic indicators of health condition (Saino and Møller, 1996), and the analysis of size of immunocompetent organs (Møller and Erritzoe, 1998). The immune system plays a pivotal role in defending an animal against attack by pathogens and parasites (Roitt et al., 1998), and in theory there are trade-offs between investments in life-history components and immune defense by hosts (Norris and Evans, 2000).

Since body mass and the visual fat score cannot reflect lipid reserves very accurately, and immunological ecology is a very recent discipline (Svensson and Skarstein, 1997), studies employing precise measurements of fat reserves and health parameters are rare (with the exception of studies in captivity and in poultry research: Klasing, 1987; Lochmiller et al., 1993). Moreover, there are considerable differences between species (Brown, 1996), and the population structure (sex ratio, young adult ratio) is also an important variable, since sex and age differences in body composition have been reported for birds (Alisauskas and Ankney, 1987; Coup and Pekins, 1999).

In this study, we use a multi-technique approach to assess body conditions in a population of hooded crows *Corvus corone cornix*. Crows are interesting passerines because of their exceptionally large size, compared to the small passerines previously studied. The aim of the study was to examine sex- and age-related profiles and to utilize a multi-technique approach to assess whether it is possible to summarize overall health conditions and immunocompetence of individuals by means of a single health state index.

MATERIALS AND METHODS

The study was carried out at Castellazzo Bormida (NW Italy, 44°51' N, 8°35' E), in the spring of 1997 and 1998, on 543 hooded crows captured with Larsen traps as part of a

\*Author to whom correspondence should be addressed. E-mail: [cucco@unipmn.it](mailto:cucco@unipmn.it)

Accepted July 2001.

provincial program of agricultural pest management. Sex was determined by gonadal examination. Age was estimated by the different upper mandible and plumage colors (Svensson, 1992). In the field, a few hours after capture, we measured (in vivo) mass and lean mass index with a TOBEC (Total Body Electrical Conductivity) apparatus, while blood samples and smears were collected for subsequent analyses. Postmortem, morphological variables and internal organs were measured, and body fat was extracted using a Soxhlet apparatus. The following data were obtained:

#### *MORPHOLOGICAL VARIABLES*

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; and (v) bill length. Tarsus and bill measurements were made to the nearest 0.1 mm, while the two other variables were measured to the nearest 0.5 mm. The four body measures (ii to v) were highly correlated with each other; thus, in all regression analyses the four parameters were compacted in an index of structural size calculated as the first factor from a PCA. Factor 1 explained 59.3% of the variance of the original variables and was highly related to them (loadings: wing = 0.814, tail = 0.793, tarsus = 0.737, bill = 0.733).

#### *LEUKOCYTE COUNTS AND ERYTHROCYTE SEDIMENTATION (ES) RATE*

We counted leukocytes and red blood cells in blood smears stained by the May-Grunwald-Geimsa method. Blood smears were scanned at 630× magnification following standard routines (Saino et al., 1995). We counted red blood cells and leukocytes classified as lymphocytes, monocytes, eosinophils, heterophils, and basophils; then we calculated the relative frequency of each family of leukocytes with respect to the total leukocyte population (relative counts) and the number of leukocytes per 10,000 red blood cells (absolute counts). To measure the sedimentation rate, 70 mL blood samples in heparinized hematocrit capillary tubes were placed in a vertical position in a refrigerated container (4 °C) for 4 h. According to Saino and Møller (1996), the sedimentation rate (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.

#### *IMMUNOGLOBULIN ASSAY*

We performed a densitometric analysis after separation of serum proteins on gel. For each animal, after wing vein puncture, about 1 mL of blood was collected in Sigma ACD and immediately stored in a bag at 4 °C to be transported to the laboratory. Blood samples were then processed on agarose gel according to the standard Hydrasys kit procedure (Sebia, France). The relative abundance of albumin and of Ig (alpha-, beta-, and gamma-globulins) was expressed as the ratio between the area of the densitometric profile corresponding to the immunoglobulin region and the total area of the densitometric profile.

#### *INTERNAL ORGAN SIZES AND BODY COMPOSITION*

We measured (i) spleen mass; (ii) bursa of Fabricius mass; and (iii) gonad mass, with a precision balance with 0.1 mg accuracy. In 201 live birds, body composition was evaluated noninvasively by measurement of electrical conductivity using the TOBEC

system. This device estimates lean body mass, since the contribution of lipid tissue to conductivity is negligible (Castro et al., 1990). We placed each individual in the detection chamber of an SA-3152 Small Animal Body Composition Analyzer (EM-SCAN Inc., Springfield, Illinois) for 5–6 s and recorded the electrical conductivity index. For each individual, the measurement was repeated three times and the average value was employed in statistical analyses. A smaller sample of 36 individuals was used to determine body composition by Soxhlet after their TOBEC indices had been measured *in vivo*. The plucked carcasses were opened and dried to a constant mass at 60 °C in a vacuum oven. This process lasted 5 to 6 days and was followed by Soxhlet extraction of the whole dried carcass for 12 h in petroleum ether. Total lean mass was calculated by summing the mass of the lean dried carcass and the water lost after initial drying (Scott et al., 1991). Statistical analyses were performed using SYSTAT (Wilkinson, 1996).

## RESULTS

### *SEX AND AGE DIFFERENCES*

Table 1 shows the results for male and female adult and subadult hooded crows. Mass and structural size were greater in males than in females and in adults than in subadults (two-way ANOVA of mass:  $F_{1,490} = 146.7$ ,  $p < 0.001$  for sex;  $F_{1,490} = 77.5$ ,  $p < 0.001$  for age, interaction  $p = \text{ns}$ ; of structural size:  $F_{1,490} = 239.8$ ,  $p < 0.001$  for sex;  $F_{1,490} = 143.8$ ,  $p < 0.001$  for age). Lean mass was also highest in males and in adults ( $F_{1,197} = 70.8$ ,  $p < 0.001$  for sex;  $F_{1,197} = 7.7$ ,  $p < 0.006$  for age, interaction  $p = \text{ns}$ ). In contrast, body fat content did not differ between age and sex categories ( $F_{1,26} = 0.359$ ,  $p = 0.55$  ns for sex;  $F_{1,26} = 0.148$ ,  $p = 0.70$  ns for age, interaction  $p = \text{ns}$ ). All individuals had a very low fat content, with a mean of 2.8% ( $\pm 1.22$  SD,  $n = 36$ ).

Most blood parameters, i.e., heterophil, eosinophil, basophil, monocyte, lymphocyte, and leukocyte numbers, albumin and gamma globulin abundance, and erythrocyte sedimentation (ES) rate, showed no age- or sex-related differences (two-way ANOVAs, all  $p > 0.05$  ns).

The spleen was heavier in subadults than in adults ( $F_{1,374} = 7.50$ ,  $p < 0.006$ ) but there were no significant differences between sexes ( $F_{1,374} = 2.86$ ,  $p = 0.09$  ns = interaction  $p$ ). The bursa of Fabricius was only observed in 57.7% of the subadults and in none of the adults. Bursa size did not differ between sexes in those subadults that had a visible organ ( $F_{1,114} = 0.984$ ,  $p = 0.323$  ns). Gonads were heavier in adults, both in females ( $F_{1,209} = 49.3$ ,  $p = 0.001$ ) and in males ( $F_{1,154} = 55.6$ ,  $p = 0.001$ ).

### *RELATIONSHIPS BETWEEN PARAMETERS*

An examination of relationships between body mass (here considered as the dependent index of body condition) and the other variables was performed by single regression analyses. In adults, none of the hematological or immunological parameters, nor the size of internal immunocompetent organs, was related to mass (all  $p = \text{ns}$ ). In subadults, the pattern was similar, with the exception of the ES rate, which was significantly inversely

Table 1  
Body condition, hematology, and immunity parameters of free-living hooded crows

Variables	Adult females		Subadult females		Adult males		Subadult males	
	$\bar{x} \pm SD$ (n)	$\bar{x} \pm SD$ (n)	$\bar{x} \pm SD$ (n)	$\bar{x} \pm SD$ (n)	$\bar{x} \pm SD$ (n)	$\bar{x} \pm SD$ (n)	$\bar{x} \pm SD$ (n)	
Structural size (Factor1)	0.017 ± 0.663 (58)	-0.709 ± 0.658 (208)			1.207 ± 0.709 (96)	0.261 ± 0.761 (132)		
Mass (g)	391.0 ± 43.4 (58)	359.3 ± 35.6 (208)			447.0 ± 43.7 (96)	404.8 ± 48.4 (132)		
Lean mass (TOBEC)	72.1 ± 17.5 (19)	68.7 ± 11.6 (103)			96.5 ± 18.6 (41)	86.1 ± 16.2 (38)		
Fat index (residuals)	5.84 ± 18.4 (19)	-15.0 ± 24.7 (103)			19.5 ± 27.6 (41)	1.6 ± 31.3 (38)		
Extracted fat (%)	2.4 ± 1.4 (8)	3.1 ± 1.6 (10)			3.1 ± 1.1 (9)	2.8 ± 0.6 (7)		
Spleen (g)	0.313 ± 0.250 (37)	0.333 ± 0.218 (173)			0.306 ± 0.171 (70)	0.429 ± 0.237 (98)		
Bursa <sup>1</sup> (g)		0.089 ± 0.167 (67)				0.115 ± 0.125 (49)		
Gonads (g)	0.181 ± 0.131 (37)	0.081 ± 0.062 (174)			0.677 ± 0.604 (64)	0.170 ± 0.224 (92)		
ES rate	0.266 ± 0.101 (18)	0.287 ± 0.120 (122)			0.254 ± 0.124 (45)	0.283 ± 0.131 (64)		
Leukocytes <sup>2</sup>	147.2 ± 136.2 (19)	133.4 ± 116.7 (115)			118.2 ± 89.8 (42)	114.5 ± 95.5 (61)		
Heterophils (%)	20.8 ± 11.8 (19)	16.8 ± 11.1 (115)			19.7 ± 14.7 (42)	20.2 ± 15.7 (61)		
Eosinophils (%)	5.7 ± 6.6 (19)	3.6 ± 3.9 (115)			3.7 ± 4.0 (42)	4.1 ± 3.9 (61)		
Basophils (%)	1.3 ± 3.0 (19)	1.8 ± 3.5 (115)			1.3 ± 2.4 (42)	1.2 ± 3.0 (61)		
Monocytes (%)	12.0 ± 27.7 (19)	3.3 ± 13.4 (115)			6.7 ± 20.9 (42)	3.7 ± 13.7 (61)		
Lymphocytes (%)	60.1 ± 29.5 (19)	71.9 ± 22.3 (115)			66.0 ± 27.8 (42)	62.6 ± 29.4 (61)		
H/L rate	0.33 ± 0.24 (19)	0.27 ± 0.27 (115)			0.34 ± 0.50 (42)	0.36 ± 0.47 (61)		
Albumin	32.1 ± 1.4 (9)	30.3 ± 1.2 (59)			30.7 ± 1.3 (26)	32.1 ± 1.4 (23)		
Gamma globulin	39.0 ± 1.3 (9)	35.2 ± 1.3 (59)			35.1 ± 1.2 (26)	36.1 ± 1.3 (23)		
Albumin/Globulins	0.95 ± 0.13 (9)	0.96 ± 0.09 (59)			0.97 ± 0.10 (26)	0.97 ± 0.13 (23)		

Notes:

<sup>1</sup> Bursa: only visible in some subadults. Statistics were calculated for 67 of 174 females, and 49 of 98 dissected males, with a visible bursa.

<sup>2</sup> Leukocytes: abundance reported as N / 10000 red blood cells.

ES—erythrocyte sedimentation; H/L—heterophils / lymphocytes.

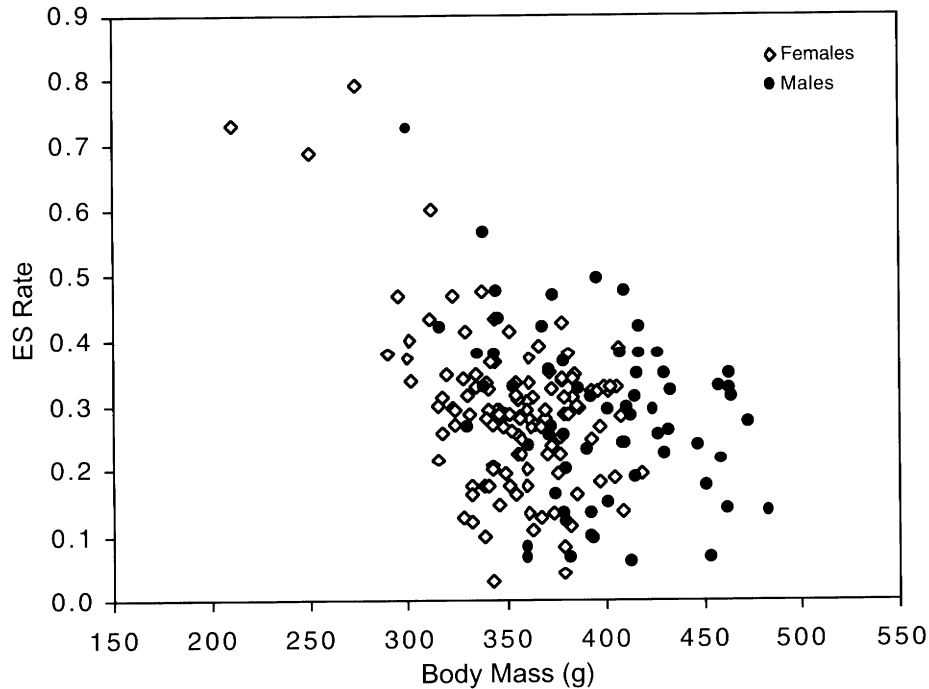


Fig. 1. Relationship between erythrocyte sedimentation rate and body mass in subadult hooded crows.

related to body mass in both sexes (males:  $r = -0.25$ ,  $p < 0.05$ ; females:  $r = -0.40$ ,  $p < 0.001$ ; Fig. 1). In the subsample of 66 subadult females with a visible bursa of Fabricius (of 174 dissected individuals), body mass was positively related to bursa size ( $r = 0.25$ ,  $p < 0.05$ ) and to ovary size ( $r = 0.18$ ,  $p < 0.02$ ).

To account for individual size, all regressions with mass as a dependent variable were also computed on size-adjusted body mass (the residual of a regression of Factor 1 on mass) as the dependent variable. The results were similar to those reported for mass alone. For the sake of brevity, they are not presented here.

An examination of the relationships between fat content, here considered as the dependent index of body condition, and the other variables, showed that in adults none of the hematological or immunological parameters, nor the size of internal immunocompetent organs, was related to fat content (all  $p = ns$ ). In subadults of both sexes, heavier individuals had a higher fat content. In subadult females, the ES rate was significantly inversely related to fat content ( $r = -0.38$ ,  $p < 0.001$ ). In subadult males, individuals with more fat had a higher number of lymphocytes ( $r = 0.45$ ,  $p < 0.02$ ) and a lower heterophil/lymphocyte ratio ( $r = -0.41$ ,  $p < 0.04$ ).

Measuring lean mass with the TOBEC apparatus only marginally improved the estimate of fat content. In a stepwise multivariate regression analysis with Soxhlet-extracted fat content as the dependent variable, and mass, structural size, and TOBEC index as independent variables, only mass was retained in the final model (complete model:  $r = 0.376$ ; stepwise selected model:  $r = 0.363$ ).

#### DISCUSSION

We investigated a large sample of birds and found that mass and body size differ between males and females, and between subadults and adults. In all four categories, the contribution of fat storage to mass (mean 3%) was negligible: since all analyzed individuals were very lean, intrapopulation differences in mass were probably due mainly to muscle protein development. Low body fat content in this species was also reported by Yom-Tov (1975), i.e., 6% of total body mass at the beginning of reproduction. The hooded crow is one of the temperate passerines with the least fat storage (Carey, 1996). The small amount of body fat reserves could be related to its synanthropic habits, with predictable sources of food (Acquarone et al., 2001a,b), and to the fact that, as an alternative to energy storage, hooded crows can hoard food and use the reserves when food is unavailable (Fjeld and Sonerud, 1988).

Because of the small percentage of fat in the body, the TOBEC method only marginally improved the estimate of fat obtained from a regression considering only mass. It is acknowledged that the TOBEC index does not always improve the estimate of lipids (Morton et al., 1991), particularly when the body has a low fat content.

An important finding of our hematological investigation comes from the comparison between subadults and adults. We found no differences in lymphocytes and heterophils with age, with the exception of a small decrease of lymphocytes in females. This is contrary to the results of the only other study on birds of comparable ages: in herring gulls *Larus argentatus*, there was a higher number of lymphocytes in adults than in immature birds (Totzke et al., 1999). The results of other studies of age-related changes seem to agree with our findings (Puerta et al., 1989; Alonso et al., 1990), but they are not strictly comparable because they considered chicks instead of subadults as the younger category.

Our study of age-related differences also considered two immune organs (the spleen and the bursa of Fabricius), and other blood parameters involved in the immune response, i.e., two plasma proteins (albumin/gamma globulin) and an index of blood cells relative to plasma volume (ES rate). The blood parameter showing the strongest difference between adults and subadults was the ES rate, a parameter that indicates that individuals are in poor condition (Merila and Svensson, 1995). In our hooded crows, the ES rates were higher in subadults: this probably indicates that younger birds face more difficulties in foraging, but it is not known if this is related to their lesser skill in finding food or to the difficulties low-ranking individuals have in gaining access to resources (Saino and De Bernardi, 1994), or if it is an effect of differential mortality (Sæther, 1990) producing a greater percentage of high-quality individuals among adults.

Recent studies have shown that it is difficult to identify a synthetic index of state of health that summarizes the different hematological and body condition parameters (Norris and Evans, 2000). For example, the significance of the leukocyte count is questionable: a high number could represent either an immunocompetent individual or a relatively poor immunocompetent individual forced to respond to an infection. Conversely, a low leukocyte number may represent poor immunocompetence, but might also reflect a lack of recent infections requiring a specific immune response from the host (Sheldon and Verhulst, 1996). On the other hand, different parts of the immune system can be differentially affected by nutrition and external stress factors; thus if different components of immune systems are effected, there is no correlated response in the measured health parameters (Norris and Evans, 2000).

In our population of hooded crows, we failed to observe a consistent pattern of the 11 measured parameters among individuals. However, a noteworthy result was the inverse relation between ES rate and mass in subadults. This suggests that ES rate indicates body mass depletion and can be used to identify individuals in poor condition. In conclusion, our study of hooded crows reveals that the four sex and age categories should be analyzed separately, and one must be cautious in choosing a single parameter to summarize the immune condition and health state of individuals.

#### REFERENCES

- Acquarone, C., Cucco, M., Li Cauli, S., Malacarne, G. 2001a. Effects of food abundance and unpredictability on body condition and health parameters: experimental tests with the Hooded Crow. *Ibis*, in press.
- Acquarone, C., Cucco, M., Malacarne, G. 2001b. Short-term effects on body condition and size of immunocompetent organs in the hooded crow. *Ital. J. Zool.*, in press.
- Alisauskas, R.T., Ankney, C.D. 1987. Age-related variation in the nutrient reserves of breeding American Coots (*Fulica americana*). *Can. J. Zool.* 65: 2417–2420.
- Alonso, J.A., Alonso, J.C., Muñoz-Pulido, R., Naveso, M.A., Abelenda, M., Huecas, V., Puerta, M.L. 1990. Hematology and blood chemistry of free-living young Great Bustard (*Otis tarda*). *Comp. Biochem. Physiol. A* 97: 611–613.
- Blem, C.R. 1990. Avian energy storage. *Curr. Ornithol.* 7: 59–113.
- Brown, M.E. 1996. Assessing body condition in birds. *Curr. Ornithol.* 13: 67–135.
- Carey, C. 1996. Avian energetics and nutritional ecology. Chapman & Hall, New York.
- Castro, G., Wunder, B.A., Knopf, F.L. 1990. Total body electrical conductivity (TOBEC) to estimate total body fat of free-living birds. *Condor* 92: 496–499.
- Coup, R.N., Pekins, P.J. 1999. Field metabolic rate of wild turkey in winter. *Can. J. Zool.* 77: 1075–1082.
- Fjeld, E., Sonerud, G.A. 1988. Food caching, cache recovery, and the use of an egg shell dump in the Hooded Crow *Corvus corone cornix*. *Ornis Scand.* 19: 268–274.
- Klasing, K.C. 1987. Influence of acute feed deprivation or excess feed intake on immunocompetence of Broiler chicks. *Poult. Sci.* 67: 626–634.
- Lochmiller, R.L., Vestey, M.R., Boren, J.C. 1993. Relationship between protein nutritional status and immunocompetence in Northern Bobwhite chicks. *Auk* 110: 503–510.
- Merila, J., Svensson, E. 1995. Fat reserves and health state in migrant Goldcrest *Regulus regulus*.

- Funct. Ecol. 9: 842–848.
- Morton, J.M., Kirkpatrick, R.L., Smith, E.P. 1991. Comments on estimating total body lipids from measures of lean mass. *Condor* 93: 463–465.
- Møller, A.P., Erritzoe, J. 1998. Host immune defence and migration in birds. *Evol. Ecol.* 12: 945–953.
- Norris, K., Evans, M.R. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behav. Ecol.* 11: 19–26.
- Pravosudov, V.V., Grubb, T.C.Jr. 1997. Energy management in passerine birds during the nonbreeding season. A review. *Curr. Ornithol.* 14: 189–234.
- Puerta, M.L., Muñoz-Pulido, R., Huecas, V., Abelenda, M. 1989. Hematology and blood chemistry of chicks of white and black storks (*Ciconia ciconia* and *Ciconia nigra*). *Comp. Biochem. Physiol. A* 94: 201–204.
- Roitt, I.M., Brostoff, J., Male, D.K. 1998. *Immunology*. London, Mosby.
- Sæther, B.E. 1990. Age-specific variation in reproductive performance of birds. *Curr. Ornithol.* 7: 251–283.
- Saino, N., De Bernardi, F. 1994. Geographic variation in size and sexual dimorphism across a hybrid zone between Carrion Crows and Hooded Crows. *Can. J. Zool.* 72: 1543–1550.
- Saino, N., Møller, A.P. 1996. Sexual ornamentation and immunocompetence in the barn swallow. *Behav. Ecol.* 7: 227–232.
- Saino, N., Møller, A.P., Bolzern, A.M. 1995. Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. *Behav. Ecol.* 6: 397–404.
- Scott, I., Grant, M., Evans, P.R. 1991. Estimation of fat-free mass of live birds: use of total body electrical conductivity (TOBEC) measurements in the studies of single species in the field. *Funct. Ecol.* 5: 314–320.
- Sheldon, B.C., Verhulst, S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* 11: 317–321.
- Svensson, L. 1992. *Identification guide to European passerines*, 4th ed. British Trust for Ornithology, Norfolk, UK.
- Svensson, E., Skarstein, F. 1997. The meeting of two cultures: bridging the gap between ecology and immunology. *Trends Ecol. Evol.* 12: 93–94.
- Totzke, U., Fenske, M., Hüppop, O., Raabe, H., Schach, N. 1999. The influence of fasting on blood and plasma composition of Herring Gull (*Larus argentatus*). *Physiol. Biochem. Zool.* 72: 426–437.
- Wilkinson, L. 1996. *SYSTAT 8.0. Statistics*. SPSS Inc., Chicag. IL.
- Yom-Tov, Y. 1975. Food of nestling crows in northeast Scotland. *Bird Study* 22: 47–51.