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SHORT NOTES

Does habitat structure affect body condition of nestlings? A case study with woodland Great Tits *Parus major*

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Abstract. A large number of studies have demonstrated that habitat characteristics have a huge impact on all aspects of animal life history. This hypothesis predicts a relationship between habitat structure and the main components of fitness and, consequently, also predicts a relationship between habitat structure and other traits indirectly related to fitness, such as condition or health. We tested whether there was any relationship between the characteristics of a pine forest in the Iberian Peninsula and the condition of Great Tit nestlings reared in that forest. The parameters used to estimate nestling condition were weight, haematocrit and the presence of haemoparasites. The results suggest that mature forests produced nestlings in better condition but with a higher prevalence of haemoparasites than young forest, possibly because mature forests are a good habitat for both the bird and the parasite vector.

Key words: forest structure, haematocrit, haemoparasites, mature forest, nestling condition, Great Tit, *Parus major*

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Characteristics of the habitat have a strong influence on food resources and shelter possibilities available for birds and other animal groups, and both food and predation are among the most important forces which constraint avian life histories (Martin 1995). Most of the studies dealing with the influence of forest structure (e.g. age, size, density of trees, etc.) on birds have found that this relationship was mediated by food quality and/or availability (e.g. Zanette et al. 2000, Suorsa et al. 2004) and could affect avian condition or breeding performance (Suorsa et al. 2003, Arriero & Fargallo 2004, Arriero et al. 2006). In general, mature forests are considered a high quality habitat for birds, usually showing better reproductive output and nestlings in better condition than young forest (Riddington & Gosler 1995, Hinsley et al. 2002).

We carried out a study in a Scots pine *Pinus sylvestris* forest trying to verify the hypothesis that habitat characteristics (specifically, maturity of the forest) have an influence on condition and parasite prevalence of Great Tit nestlings reared in nest-boxes in that habitat. Parasites are one of the main selective forces in avian life-histories (Hamilton & Zuk 1982, Loye & Zuk 1991, Goater & Holmes 1997, Merino et al. 2000). The presence and intensity of infestations may reflect the ability of the host to cope with infections and could be used as indexes of phenotypic quality. We have used weight and the level of haematocrit to estimate condition in birds. Weight, either absolute or relative to body size, is the most commonly used parameter to express condition in birds (e.g. Naef-Daenzer et al. 2001). Haematocrit, the proportion of blood volume occupied by packed red blood cells, reflects the efficiency in the transfer of oxygen to the tissues (Ots et al. 1998). Factors like age, sex, reproductive status, environmental conditions, parasitism or nutritional status have been thought to have an influence on haematocrit (Fair et al. 2007 and references therein). Although some studies cast doubt on the suitability of haematocrit as an index of condition (Dawson & Bortolotti 1997a, b), a large number of recent studies support its use to estimate condition and nutritional status (e.g. Sánchez-Guzmán et al. 2004, Simon et al. 2004, Villegas et al. 2004).

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Our hypothesis that maturity of the forest (i.e., quality of the habitat) has an influence on condition of Great Tit nestlings has three clear predictions: nestlings reared in mature forest (1) will be heavier, (2) will show higher levels of haematocrit, and (3) will show lower levels of parasitization than nestlings reared in young forest.

Field work was conducted in the northeast of Navarre, Spain (42°39′–42°49′N, 0°54′–1°9′W), in three areas of managed Scots pine forest. Twelve forest plots were selected, trying to maximize tree size variability among plots. All plots were part of large forest areas and were not close to the edges of the forest. Every plot was a 25 m-radius circle separated from the closest plot by at least 500 m. A total of 300 nest-boxes were placed in the study area in February 2002, specifically 25 nest-boxes, separated 8–13 m from each other, in each forest plot. Between April and July 2003, all nest-boxes were checked every 15 days and nest-boxes with eggs were checked every 4–5 days. Prior to the breeding season nest-boxes had been checked and cleaned.

Great Tit nestlings reared in nest-boxes were ringed, measured (weight to the nearest 0.25 g and tarsus length to the nearest 0.01 mm) and blood sampled when they were about 14 days old (range 13–16). A drop of blood was smeared on individually marked microscope slides, air dried, fixed in absolute ethanol and later stained with Giemsa dye. Smears were used to detect parasite infections. Once parasites were found, they were identified and counted. To detect parasites, one-half of the smear was entirely scanned at 200x along its longitudinal axis and the other half under oil at 1000x (Merino & Potti 1995). As we have detected very low prevalence, we have not considered the number of parasites. Nine μl blood samples were taken in heparinized microhaematocrit capillary tubes and centrifuged at 11500 rpm for 3.3 min in a Microspin portable centrifuge. The length of the tube occupied by erythrocytes and the length of the tube occupied by all blood components were measured to the nearest 0.01 mm using a graduate magnifier in order to calculate the haematocrit. A total of 67 Great Tit nestlings belonging to 10 broods were ringed, measured and blood sampled.

Vegetation was studied only inside the plots. All trees (≥ 10 cm diameter at breast height (DBH) and > 3 m tall), either dead or alive, were counted and their height, DBH, and state of decay recorded. Height was measured with a clinometer. Decay of living trees was classified according to Carey & Healy (1981). Vegetation profiles were made at 16 points, half of which were placed 10 m and the other half 20 m from the centre of the plot, on eight axes that crossed the centre at 45° angles to each other. At each point, plant species were identified and measured. This allowed to estimate the percentage of the plot covered by herbaceous vegetation (< 0.5 m high) and shrubs (0.5–3 m high).

In order to avoid possible biases in the data due to the inclusion of information from second clutches only in some plots, we have included the information of clutches that, because of time constraints, could only be first clutches from different pairs. Haematocrit value for every plot represents the mean value of haematocrit for all nestlings within that plot. A plot was characterized as parasitized when at least one of the nestlings reared in that plot was parasitized. A principal components analysis (PCA) was carried out on eight vegetation variables (Table 1) to find the main components summarizing the vegetation structure in pine forests. Then, the relationship between the resulting factors and the characteristics of Great Tit nestlings was investigated. All statistical analyses, performed with the Stastistica (StatSoft 2001) program, were two-tailed with a significance level of 0.05.

The PCA results showed two factors with an eigenvalue higher than one which explained 74% of the variance (Table 1). Factor PC1 was positively related to the number of trees and the degree of tree decay and negatively related to the percentage of bush cover. Therefore, PC1 would represent the openness of the forest, with open forests

Table 1. Loading factors, eigenvalues and cumulative proportion of variance explained resulting from the principal component analysis of vegetation structure variables. Significant variables for each principal component are underlined.

Vegetation structure variables	PC1	PC2
Number of trees	0.885	-0.003
DBH	0.489	0.780
Height of trees	0.640	0.672
Decay state of trees	0.934	0.083
Volume of dead wood	0.630	0.614
Decay state of dead wood	0.665	0.204
% herbaceous vegetation	0.385	-0.731
% shrub vegetation	-0.754	-0.185
Eigenvalue	4.514	1.402
% variance explained	56.428	17.526
Σ % variance explained	56.428	73.954

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in one extreme of the gradient (characterized by high percentage of bushes) and close forests in the opposite extreme (characterized by high density of trees). Factor PC2 was positively related to the DBH of the trees and negatively related to the percentage of herbaceous cover. Then, PC2 would represent the maturity of the forest, with big trees and no gaps in one extreme of the gradient (mature forests) and small trees and gaps in the other extreme (young forests).

Great Tits bred in only 8 out of the 12 study plots. Therefore, the following analyses will show a sample size of 8 instead of 12. We found a marginally non-significant relationship in the expected direction between the weight of Great Tit nestlings and PC2 (Spearman correlation, $n = 8$, $r = 0.69$, $p = 0.058$). However, the relationship did not improve when body mass was controlled for tarsus length (an estimate of body size) in a partial correlation ($r = 0.51$, $p = 0.10$). The relationship between PC1 and body mass was far from significant ($r = -0.024$, $p = 0.95$). The haematocrit of Great Tit nestlings was positively related to PC2 ($r = 0.881$, $p = 0.004$) (Fig. 1), but there was no significant relationship between PC1 and haematocrit level ($r = 0.071$, $p = 0.87$). Breeding phenology could be a confounding variable if it were related to habitat structure or nestling condition, but this was not the case in our study ($-0.275 \leq r \leq 0.371$, $p \geq 0.37$ in all Spearman correlations between hatching date and PC1, PC2, haematocrit, tarsus length and weight).

Leucocytozoon sp. was the only haemoparasite found in the sample, and was detected in 5 out of the 67 chicks analyzed, a rather low prevalence (7.5%). PC1 values did not differ significantly between parasitized and non-parasitized plots (Mann-Whitney U-test, for both parasitized and non-parasitized $n=4$, $Z = -0.29$, $p = 0.77$), but parasitized plots showed higher values of PC2 than non-parasitized ones ($Z = 2.31$, $p = 0.021$) (Fig. 2).

In agreement with our prediction, Great Tit nestlings showed higher levels of haematocrit when reared in mature forest plots (with large trees and small gaps) than in young forest plots. This result, together with the non-significant trend for heavier nestlings in mature forests, suggests that Great Tit nestlings reared in high quality habitat (mature forest) indeed showed a better condition than nestlings reared in low quality habitat (young forest). Since the amount of food received by chicks has a positive effect on their haematocrit (Merino & Potti 1998, Moreno et al. 1999, Potti et al. 1999), the relationship found between nestlings haematocrit and maturity of the forest could be mediated by a higher availability of food resources in mature forest plots (Riddington & Gosler 1995, Hinsley et al. 2002).

The prevalence of haemoparasites in chicks was very low, although it should be taken into account that age is a factor influencing the prevalence and intensity of infections (Allander

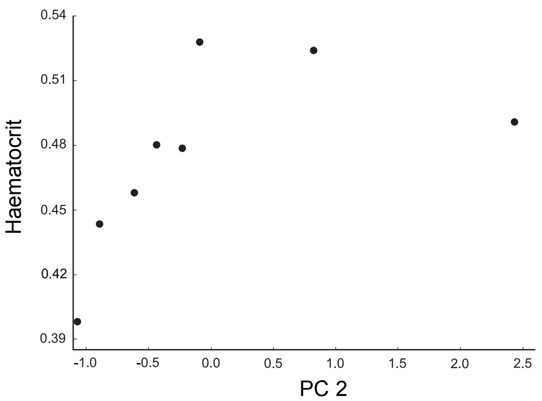

Fig. 1. Relationship between mean haematocrit of Great Tit nestlings per forest plot (n = 8) and PC2 factor (associated positively to DBH of trees and negatively to proportion of herbaceous cover). Statistical values in the text.

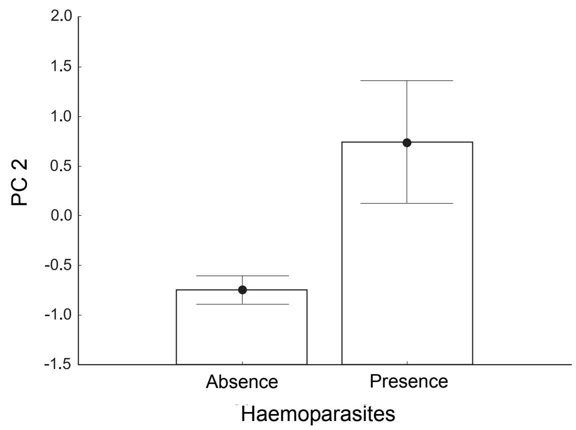

Fig. 2. Mean values (± SE) of the PC2 factor (associated positively to DBH of trees and negatively to proportion of herbaceous cover) in parasitized and non-parasitized forest plots. A plot was defined as parasitized when at least one of the chicks reared in that plot was parasitized. Statistical values in the text.

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& Bennet 1994, Merilä et al. 1995, but see Merino & Potti 1995). Moreover, the prepatent period of the blood parasites detected in Great Tit nestlings varies among the different genera: it is only 5–6 days for *Leucocytozoon* sp. but 14 days for *Haemoproteus* sp. and *Plasmodium* sp. (Fallis & Bennett 1961, Atkinson & Van Riper III 1991). Therefore, the absence of certain parasites in the smears does not imply the absence of these parasites in the area or the immunity of the chicks, but maybe the short period of time between parasite inoculation and blood collection.

Contrary to our expectations, a higher prevalence of parasites was found in nestlings reared in mature forest. *Leucocytozoon* sp., the only parasite detected, is transmitted by haematophagous flies that require a water stream for reproduction and atmospheric humidity (Werner & Pont 2003). It is possible that young forests present higher solar radiation and become drier environments than mature forests. In accordance to this suggestion, Krone et al. (2001) found a low prevalence of *Leucocytozoon* sp. vectors in young pine plantations due to dry conditions for these flies.

Great Tit nestlings reared in mature pine forests showed higher parasite prevalence and also higher levels of haematocrit than nestlings reared in young forests. It is unlikely that high levels of haematocrit had been caused by the parasitic infection since severe infections caused by *Leucocytozoon* sp. have been described as a cause of anemia (Merino & Potti 1998, Moreno et al. 1999, Potti et al. 1999). However, infections are not always followed by a noticeable decrease in haematocrit (Atkinson & Van Riper III 1991, Fair et al. 2007 and references therein). The most likely explanation for the relationship between forest maturity and both haematocrit level and parasite prevalence in Great Tit nestlings seems to be that mature forests are suitable environments for both haemoparasite vectors and birds (Arriero et al. 2004).

It is important to underline that, as we did not capture adult birds, we do not know if there was a relationship between chick and parent quality or between adult and forest quality. In any case, we have found a relationship between habitat and chick characteristics, either direct or mediated by the quality of the parents.

To summarize, this study suggests that the relationship between quality of the habitat and phenotypic quality of individuals is not simple, and can be altered in many different ways due to habitat effects on different species, and how these species interact with each other. In any case, the structure of the forest seems to exert a strong influence on nestling fitness.

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STRESZCZENIE

[Wpływ struktury środowiska na kondycję piskląt bogatki]

Badano zależność między charakterystyką lasów sosnowych na półwyspie Iberyjskim a kondycją piskląt bogatki wychowanych w tych lasach. Wyznaczono łącznie 12 powierzchni — okręgów o promieniu 25 m, w których rozwieszono po 25 skrzynek lęgowych w każdym. Kondycja ptaków oceniana była u ok. 14 dniowych piskląt poprzez ich ciężar ciała, hematokryt i obecność pasożytów krwi. Drzewostan każdej powierzchni leśnej był opisywany (m. in. liczba drzew, ich grubość, wysokość, żywotność itd.), zaś rośliny zielne charakteryzowane były w 16 punktach ułożonych dość równomiernie na całej powierzchni.

Wszystkie dane dotyczące drzewostanów zredukowane zostały przy użyciu analizy składowych głównych (Tab. 1).

Stwierdzono, że ciężar ciała piskląt wzrastał wraz ze wzrastającą dojrzałością drzewostanów, choć jest zależność ta była marginalnie nieistotna statystycznie. Podobną, istotną zależność stwierdzono dla hematokrytu (Fig. 1). Natomiast pasożyty krwi u piskląt (wyłącznie *Leucocytozoon* sp.) częściej stwierdzano w lasach starszych, z grubszymi drzewami i większą zwartością drzewostanu (Fig. 2).

Uzyskane wyniki sugerują, że w dojrzałych, starszych lasach sosnowych pisklęta opuszczają gniazda w lepszej kondycji, ale także częściej są narażone na pasożyty krwi niż w lasach młodych. Jest to prawdopodobnie związane z faktem, że w starszych lasach środowisko jest lepsze zarówno dla ptaków, jak i wektorów pasożytów.

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