

Ecological factors influencing disease risk in Eagle Owls *Bubo bubo*

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In this study we assessed whether local habitat features and host population density influenced disease risk in Eagle Owl *Bubo bubo* fledglings. Measures of immune defence (concentrations of circulating white blood cells), prevalence of three parasite types (a blood parasite *Leucocytozoon ziemanni*, an insect *Carnus haemapterus*, and a tick *Rhipicephalus* sp.) and total number of parasite species were used to quantify disease risk. We tested the hypotheses that disease risk in fledglings was higher in nests located in areas with higher length of and proximity to watercourses (as a higher abundance and viability of parasites and vectors occur in wetter areas), higher cover of forest (as forest moistness and humidity can favour higher vector and parasite proliferation), higher habitat diversity (as environmental heterogeneity increases the pool of potential vectors and parasites) and higher local owl population density (as disease transmission might be density-dependent). The clearest relationship was with the proximity of freshwater, although the other hypotheses were also partially supported. Concentrations of white blood cells, the number of parasite species and, weakly, the prevalence of *Carnus haemapterus* were all higher in nests closer to watercourses. The prevalence of blood parasites increased with the cover of forested areas. Fledglings from nests located in more diverse habitats had higher white blood cell concentrations and showed higher prevalence of blood parasites. Finally, local host population density was positively correlated with the prevalence of blood parasites. The results suggest the existence of complex and interrelated links between ecological parameters and three different measures of disease risk, and highlight the importance of immunological approaches to assess disease risk at an intraspecific level.

Parasites and infectious diseases play an important role in host evolution and population regulation, as they reduce reproductive success and increase mortality (Møller 1997). Recent studies have found that social, ecological and life-history factors are important in determining disease risk (Arneberg 2001, Møller *et al.* 2001, Nunn 2002, Semple *et al.* 2002, Nunn *et al.* 2003a, 2003b). Identifying factors that influence disease risk in natural populations is crucial in understanding host ecology (Mendes *et al.* 2005) and in establishing general principles for managing threatened wildlife populations (Nunn *et al.* 2003b). Nevertheless, the sources of variability in the occurrence of diseases are still poorly known (Nunn *et al.* 2003a, 2003b).

Three main approaches have been commonly employed to quantify disease risk in both within- and between-species comparisons (Nunn 2002): measuring differences in parasite species richness (Nunn *et al.* 2003a, Vitone *et al.* 2004), measuring differences in parasite burden (Arneberg 2001) and using the host immune response as a measure of disease risk (Møller 1998, Møller *et al.* 2001, Nunn 2002, Semple *et al.* 2002, Nunn *et al.* 2003b, Cotter *et al.* 2004). The last is based on the assumption that hosts suffering more from diseases and parasites should increase their immune response to resist them. This adjustment ought to be precise, as the maintenance of the immune defence is costly (Sheldon & Verhulst 1996, Norris & Evans 2000, Nunn 2002). The abundance of parasites in a host population is not a reliable index of parasite pressure because host defence can have a strong influence on parasite abundance. For

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example, a host can be under high parasite pressure, yet have few parasites, because it has invested heavily in defence (Moyer *et al.* 2002). Thus, the immunological approach is considered to provide a more useful measure of disease risk in comparison with methods based on direct quantification of parasites, as the other methods only assess disease risk that remains after host counterstrategies, such as immune defence, have been implemented (Nunn 2002). On the other hand, decoupling parasite pressure from defence by experimentally suppressing defence (e.g. using immunosuppressive agents) without influencing other aspects of host physiology is difficult (Norris & Evans 2000, Moyer *et al.* 2002, Adamo 2004). The use of immune system variation for disease risk assessment has been mainly developed for interspecific comparisons, and analyses of the extent to which different ecological conditions affect disease risk of individuals within species are scarce (Tella *et al.* 2001, Cotter *et al.* 2004, Martin *et al.* 2004). This last perspective may provide new insights into the mechanisms underlying patterns of disease risk observed between species (Nunn *et al.* 2003b).

Here we assess whether different ecological factors are associated with disease risk in fledgling Eagle Owls *Bubo bubo*. Unlike most previous comparable studies (e.g. Møller 1998, Tella *et al.* 1999, Nunn 2002, Semple *et al.* 2002, Nunn *et al.* 2003a, 2003b), we adopted a multiple approach, using measures of immune defence combined with the presence or absence of three parasite types (a blood parasite *Leucocytozoon ziemanni*, a parasitic insect *Carnus haemapterus*, and a tick *Rhipicephalus* sp.) and the count of each of these parasite species present. We used measures of immune response, specifically concentrations of circulating white blood cells (WBCs), as indicators of disease risk (Møller 1998, Nunn 2002, Semple *et al.* 2002, Nunn *et al.* 2003b). Most comparative studies have used immune system parameters from healthy individuals, for example from captive populations (Nunn 2002, Semple *et al.* 2002), assuming that interspecific variations in baseline host defence result from an evolutionary adjustment related to different levels of disease risk (Nunn 2002, Semple *et al.* 2002, Nunn *et al.* 2003b). By contrast, our approach involves a single species with individuals exposed to different suites of parasites and pathogens in the wild, so higher levels of circulating WBCs are likely to be the consequence of immune system response to current active infections and parasitism (e.g. Roitt *et al.* 2001, Davis *et al.* 2004). The maintenance of immune defence entails trade-offs with

other life-history traits (Sheldon & Verhulst 1996), leading to the assumption that individuals in prime condition are better able to develop enhanced immune systems to ward off possible diseases (Møller *et al.* 1998, Møller & Petrie 2002). Thus, predicting directional changes in WBC concentrations is difficult, and it could be that higher concentrations reflect either the healthy status of uninfected individuals or a host immune response to current active infection (Davis *et al.* 2004).

We tested the hypothesis that disease risk was related to both habitat features and host population density in fledgling Eagle Owls. In particular, we assessed (1) whether disease risk was higher for nests in areas with greater length and number of watercourses, due to higher abundance and viability in wetter areas of several species of parasites and vectors (Little & Earlé 1995, Møller 1998, Moyer *et al.* 2002, Semple *et al.* 2002, Mendes *et al.* 2005, Nunn *et al.* 2005); (2) whether disease risk was greater in fledglings in nests in areas of higher forest cover, due to higher moistness and humidity in forests in comparison with open areas (Tella *et al.* 1999, Garvin & Greiner 2003); (3) whether disease risk was higher in nests located in areas with higher habitat diversity, because heterogeneous habitats are likely to contain a large number of different microhabitats, increasing the potential pool of parasites or pathogens (Gregory 1990, Nunn *et al.* 2003a, 2004, Vitone *et al.* 2004) and (4) whether fledglings from nests located in areas with high owl population density suffer higher disease risk due to the higher horizontal disease transmission rates between hosts in such conditions (Anderson & May 1979). We also assessed the relationship between parasite load and WBC concentrations.

METHODS

Study area

The study area, Toledo province in central Spain (39°47'N, 4°04'W), covered 2400 km² and has a meso-Mediterranean climate. Mean temperatures in this region range from 5 °C in January to 26 °C in July and average rainfall is 300–400 mm, with spring and autumn peaks. The area is extensively cultivated with olive groves *Olea europaea*, vineyards *Vitis vinifera*, barley *Hordeum vulgare* and wheat *Triticum* spp. Holm oaks *Quercus ilex* dominate the less intensively used areas, whereas the most degraded zones are dominated by esparto grass *Stipa tenacissima* or Mediterranean scrubland mainly composed of *Quercus*

ilix shrubs, *Cistus ladanifer* and *Retama sphaerocarpa*. Other habitats include streams with riparian vegetation and recent pine *Pinus* spp. plantations.

Parasite characteristics

We focused on three parasite types established in Eagle Owl fledglings: a protozoan blood parasite *Leucocytozoon ziemanni*, an insect *Carnus haemapterus*, and a tick *Rhipicephalus* sp. We chose these parasite species because they are relatively common parasites of Eagle Owl fledglings and their presence is easy to assess during systematic nest monitoring. *Leucocytozoon ziemanni* is the only *Leucocytozoon* species so far recorded in owls. The species has a worldwide distribution, infects a great variety of owl species and is transmitted by ornithophilic black flies (Diptera: Simuliidae). Although *L. ziemanni* is usually considered non-pathogenic, reduced reproduction and increased mortality have been recorded when trophic conditions for the host are unfavourable (Remple 2004). *Carnus haemapterus* is a small (2 mm) blood-sucking fly that parasitizes nestlings of a wide variety of bird species across a broad geographical range (Valera *et al.* 2003, 2004). The species overwinters as pupae in the nest debris and after emergence adults seek a suitable host. Once flies are established on a host they lose their wings and move to the axillae of nestlings (Valera *et al.* 2003). Nestlings both of Eagle Owls (J. Ortego pers. obs.) and of other species appear to become free of this parasite close to fledging and it has never been recorded parasitizing adults of any bird species (Valera *et al.* 2003). The consequences of infestations by this ectoparasite are unclear as some studies have described certain negative effects (mainly related to blood loss) whereas others have found none (Valera *et al.* 2004). *Rhipicephalus* sp. is a tick that predominantly parasitizes mammals, although it has also occasionally been recorded on birds, including Eagle Owls (Silva *et al.* 2001). Fledgling Eagle Owls probably become parasitized as a consequence of the long time that they spend in contact with the ground and through the consumption of prey species usually parasitized by this tick, such as rabbits *Oryctolagus cuniculus* and rats *Rattus* spp. (Silva *et al.* 2001). As with other ticks, *Rhipicephalus* may cause blood loss and can transmit certain pathogens, such as *Borrelia* spp. (Singh & Girschick 2003), to the infested host. Neither the direct nor the indirect effects of *Rhipicephalus* infestations have been studied in detail on avian species, but they appear to have minor consequences on fitness (Singh & Girschick 2003).

Field data collection

From March to early June 2004 we monitored 27 successful nests of Eagle Owls in the study area. Nests were visited at least twice during the nesting period. During a first visit (when chicks were around 20 days old) we calculated the age of the chicks according to their feather development using data from 11 nests containing chicks with known hatching dates (Marchesi *et al.* 2002, Penteriani *et al.* 2005). During this visit we also recorded the prevalence of *Carnus haemapterus*, as its presence within an infested brood declines during the brood-rearing period and chicks become parasite-free close to fledging (Valera *et al.* 2003). We searched for *Carnus haemapterus* under the wing surface, where these ectoparasites congregate (Valera *et al.* 2003). The second visit was carried out when fledglings were around 40 days old. We collected blood samples, measured fledglings and recorded the presence or absence of blood parasites and ticks. Presence of *Rhipicephalus* sp. was assessed by examining the eyelids and areas of bare skin around the eyes and the bill, where ticks most commonly congregate (Silva *et al.* 2001).

We collected blood by puncturing the brachial vein and transferred it to heparinized microcapillary tubes. Immediately, a drop of blood was smeared on four individually marked microscope slides. Each smear was rapidly air-dried, fixed with absolute ethanol and later stained in the laboratory with Giemsa's solution (1 : 10) for 45 min. One to eight microcapillary tubes were sealed with plasticine and stored in crushed ice until they were centrifuged at 14 000 g for 10 min in a microcapillary centrifuge. Haematocrit was determined by means of a calliper to the nearest 0.01 mm and the average value across tubes from the same chick was calculated.

We weighed fledglings with a 2.5-kg Pesola scale with precision of 10 g, and measured length of tarsus, hind claw and bill using callipers to the nearest 0.01 mm. To determine an index of body size, we carried out a principal component analysis using as input variables the three biometric measurements taken ($n = 82$ chicks). We used a multiple trait index because this is expected to provide a more reliable indicator of structural size than any single measure alone (Green 2001). The first principal component (PC1) accounted for 81.67% of the variance and was adopted as a synthetic index of body size for fledglings at 40 days. A physical condition index was then calculated for each bird using the residuals from a regression of body mass on the scores from PC1. This regression was positive and statistically significant

(body mass = $1352.97 + 124.17(\text{PC1})$; $F_{1,80} = 90.35$, $r^2 = 0.53$, $P < 0.001$).

Determination of WBC concentrations and prevalence of blood parasites

Smears were scanned at 1000 \times magnification under oil immersion using standard routines (Merino *et al.* 1999). In each smear we counted 100 WBCs and recorded the number of fields scanned during the WBC count as well as the number of red blood cells per field. This allowed us to estimate overall WBC concentrations by calculating the number of WBCs observed per 2000 erythrocytes (Hörak *et al.* 1999, Merino *et al.* 1999, Davis *et al.* 2004). The presence of blood parasites in the blood smears was ascertained during leucocyte counts. When no gametocyte was detected during this examination (around 40 fields) another examination at 400 \times magnification was carried out for 5 min to avoid false negative records. These protocols have been previously reported to be highly repeatable (Allander & Sundberg 1997, Moreno *et al.* 1998). Both WBC counts and determination of prevalence of blood parasites were carried out by F.E., who had no information about the individual birds except ring number.

Habitat features and Owl population density

We measured five habitat variables related to vegetation cover, presence of watercourses and habitat diversity. These variables were measured within a 1500-m radius of the nest, an area of 7.07 km². This scale reflects the main hunting area for parent Eagle Owls during the breeding season and is assumed to be representative of the habitats around the nest (Marchesi *et al.* 2002, Ortego & Díaz 2004, Sergio *et al.* 2004). We determined the distance and length of watercourses from 1 : 25 000 topographic maps of Spain (IGN). Nest-sites were incorporated into a Geographic Information System (GIS) and afterwards vegetation cover and habitat diversity (Simpson's index, calculated as: $1 - \sum p^2$, where p is the proportion of each habitat type) were measured from digitized 1 : 100 000 CORINE Land Cover maps using Arc-View software (ArcView 3.2, ESRI, Redlands, CA, USA). The 14 land-use types provided by CORINE Land Cover maps were grouped into two categories: (1) forested habitats, which grouped (a) forests of evergreen sclerophyllous and Lusitanian oaks, (b) transitional woodland–scrubland, (c) conif-

erous forest, (d) other broad-leaved tree plantations, (e) agro-forestry areas; and (2) open habitats, the sum of (a) non-irrigated arable land, (b) land occupied mainly by agricultural uses with some areas of natural vegetation, (c) vineyards, (d) olive groves, (e) mixture of perennial crops, (f) mixtures of annual and perennial crops, (g) saline areas, (h) low-density scrub and scrubland and (i) high scrubland formations of medium to high density.

The local population density of Eagle Owls in the vicinity of each nest was defined as the number of other territories within 1500 m. We censused Eagle Owl pairs by means of intensive nest searching, listening to spontaneous vocalizations, visiting the area around potential nest-sites or perch sites to look for moulted feathers, fresh pellets and prey remains, and eliciting territorial calls by means of playbacks of conspecific vocalizations (Marchesi *et al.* 2002). Eagle Owl lifespan in the wild is around 15–20 years and territories are usually highly stable (Snow & Perrins 1998, Penteriani *et al.* 2004). In this way, host population density can be considered relatively constant and variation in densities through time is likely to be small relative to variation between locations.

Statistical analyses

We used forward stepwise regression procedures to identify those ecological parameters that best explained the following dependent variables: overall WBC concentrations, prevalence (presence/absence) of blood parasites, carnid flies and ticks, and number of parasite species found (0–3). Each of these dependent variables was modelled separately in terms of the five predictors hypothesized to be related to disease risk: distance to the nearest watercourse, length of watercourses within 1.5 km, habitat diversity index, cover of forested habitats within 1.5 km, and number of other Eagle Owl territories within 1.5 km. To reduce collinearity problems, the cover of open areas was not included into the analyses given that it showed a strong negative correlation ($r < -0.99$) with the cover of forests (Green 1979). All measures taken from the chicks were averaged across the brood and we took the nest as the statistical unit throughout the analyses. When the dependent variable was overall WBC concentrations we used multiple regression analyses with normal distribution of errors and identity link function (StatSoft 1996). To analyse factors affecting the presence or absence of each parasite, we used logistic regression analyses with binomial errors and logit-link function (Crawley 1993). In order to avoid

Table 1. Forward stepwise multiple regression model (normal error and identity link function) of total WBC concentrations in fledgling Eagle Owls ($n = 27$ nests) testing the effect of ecological parameters, chick condition and laying date. Only distance to watercourse and habitat diversity were retained in the final model.

| Source of variation | Parameter estimate | \pm se | Wald | P |
|-------------------------|--------------------|----------|-------|---------|
| Constant | 30.75 | 3.72 | 68.29 | < 0.001 |
| Distance to watercourse | -4.16 | 2.10 | 3.91 | 0.048 |
| Habitat diversity | 10.79 | 5.05 | 4.56 | 0.033 |

information loss on the sample size from which the prevalence is estimated, we used the number of parasitized chicks within the nest as the response variable and the total number of chicks from that nest as the binomial denominator (Crawley 1993, Tella *et al.* 1999). Finally, to analyse the number of parasite species present within each nest we used a multiple regression analysis with Poisson distribution and log-link function (Crawley 1993, Calvete *et al.* 2004).

Individuals in prime condition are better able to develop enhanced immune systems to ward off possible diseases than individuals in poor condition (Møller *et al.* 1998, Møller & Petrie 2002, see Introduction). By contrast, an individual can be under high parasitism pressure yet have few parasites, because it has invested heavily in defence (Moyer *et al.* 2002). In this sense, we controlled for host condition, estimated by haematocrit and residual body mass, entering these variables as additional predictors into the analyses of overall WBC concentrations, prevalence of parasites and number of parasite species (Calvete *et al.* 2004, Cotter *et al.* 2004). Finally, we included laying date in our analyses, as parasite populations and infection risk increase as the reproductive season progresses (Merino *et al.* 2000) and immunocompetence usually decreases with breeding date (Sorci *et al.* 1997, Dubiec & Cichon 2001).

Finally, we assessed whether WBC counts were associated with the prevalence of parasites in individual fledglings by modelling WBC concentrations in an ANCOVA (Statsoft 1996). The presence or absence of *Leucocytozoon ziemanni*, *Carnus haemapterus*, and *Rhipicephalus* sp. were added as independent factors, and haematocrit and residual body mass were included as covariates. Quadratic terms were also entered to account for potential non-linear relationships. All statistical tests are two tailed with the significance level set at $P < 0.05$. Some variables explaining a smaller amount of variance ($P < 0.10$) were retained in the models but they were always interpreted as marginally significant effects. All statistical procedures were performed with STATISTICA software (StatSoft 1996) and GLIM (Crawley 1993).

RESULTS

The average nearest-neighbour distance in the study population was 1369 m (sd = ± 1620 , range = 150–8250, $n = 115$ nests), among the lowest reported to date for this species (Marchesi *et al.* 2002, Ortego & Díaz 2004). The average number of other territories within 1500 m of the studied nests was 1.96 (sd = ± 1.40 , range = 0–5, $n = 27$ nests). Overall WBC concentrations were positively associated with habitat diversity and negatively associated with distance to the nearest watercourse. However, we found no effect of chick condition parameters (haematocrit and residual body mass) or laying date on WBC concentrations (Table 1). The presence of *Leucocytozoon ziemanni* was positively correlated with habitat diversity, cover of forest and local owl population density and showed a marginal positive association with laying date. The presence of *Carnus haemapterus* was positively associated with laying date and to a lesser extent with length of watercourses. None of the variables explained significant variation in the presence of *Rhipicephalus* sp. (Table 2). The number of parasite species present in a brood increased with increasing length of watercourses and laying date (Table 3). No quadratic terms were significant in any analysis ($P > 0.1$ in all cases). Neither chick condition parameters (residual body mass: $F_{1,82} = 0.10$, $P = 0.749$; haematocrit: $F_{1,82} = 0.23$, $P = 0.633$) nor prevalence of *Leucocytozoon ziemanni* ($F_{1,82} = 0.23$, $P = 0.636$), *Carnus haemapterus* ($F_{1,82} = 0.19$, $P = 0.663$), and *Rhipicephalus* sp. ($F_{1,82} = 0.83$, $P = 0.336$) affected overall WBC concentrations.

DISCUSSION

Factors influencing disease risk

Disease risk was associated with several of the ecological parameters examined, most frequently and strongly with proximity to freshwater. Habitat heterogeneity, forest cover and host population density were also significant predictors of some of

Table 2. Forward stepwise multiple regression models (binomial error and logit-link function) of the presence or absence of *Leucocytozoon ziemanni*, *Carnus haemapterus*, and *Rhipicephalus* sp. in fledgling Eagle Owls ($n = 27$ nests).

| Source of variation | Δ deviance | <i>df</i> | Parameter estimate | \pm se | <i>P</i> |
|-----------------------------------|-------------------|-----------|--------------------|----------|----------|
| <i>Leucocytozoon ziemanni</i> | | | | | |
| Null model | 84.95 | 26 | | | |
| Final model | 59.09 | 22 | | | |
| Constant | | | -1256.00 | 677.40 | |
| Owl population density | 7.45 | 1 | 1.64 | 0.63 | 0.006 |
| Habitat diversity | 8.49 | 1 | 5.40 | 2.14 | 0.004 |
| Cover of forest | 6.20 | 1 | 2.03 | 0.89 | 0.013 |
| Laying date | 3.72 | 1 | 0.03 | 0.02 | 0.054 |
| <i>Carnus haemapterus</i> | | | | | |
| Null model | 35.59 | 26 | | | |
| Final model | 15.68 | 24 | | | |
| Constant | | | -4957.00 | 1956.00 | |
| Laying date | 16.30 | 1 | 0.13 | 0.05 | < 0.001 |
| Length of watercourses | 3.61 | 1 | 10.89 | 8.06 | 0.057 |
| <i>Rhipicephalus</i> sp. | | | | | |
| Null model | 35.33 | 26 | | | |
| Final model | 35.33 | 26 | | | |
| No variable retained in the model | | | | | |

Table 3. Forward stepwise multiple regression model (Poisson distribution and log-link) of the number of parasite species in fledgling Eagle Owls ($n = 27$ nests).

| Variable | Δ deviance | <i>df</i> | Parameter estimate | \pm se | <i>P</i> |
|------------------------|-------------------|-----------|--------------------|----------|----------|
| Null model | 19.58 | 26 | | | |
| Final model | 8.32 | 24 | | | |
| Constant | | | -640.00 | 287.20 | |
| Length of watercourses | 6.36 | 1 | 3.95 | 1.78 | 0.012 |
| Laying date | 4.89 | 1 | 0.02 | 0.01 | 0.027 |

the measures of disease risk examined. These results support previous findings from cross-species studies, suggesting that patterns among species can also be detected in more detailed analyses at the species level. Furthermore, our results indicate that the use of immune system parameters is an adequate tool for disease risk assessment at the intraspecific level (Møller 1998, Nunn 2002, Semple *et al.* 2002, Nunn *et al.* 2003b).

Freshwater availability and habitat moistness, estimated by the proximity to watercourses, were positively associated with WBC concentrations, indicating that fledgling Eagle Owls from nests located in the vicinity of watercourses probably suffered higher disease risk (Table 1). Indeed, the number of species parasitizing Eagle Owl broods and to a lesser extent the prevalence of *Carnus haemapterus* increased with the length of watercourses around the nest (Tables 2 & 3). These findings are

consistent with the fact that numerous pathogens, including ecto- and endoparasites, need water and/or high humidity to complete certain phases of their life cycles (egg development, free-living larval stages, etc.; Moyer *et al.* 2002) so that the absence of freshwater can result in the interruption of their transmission and viability (Møller 1998, Semple *et al.* 2002, Mendes *et al.* 2005, Nunn *et al.* 2005). Furthermore, availability of freshwater can increase population sizes and, thus, biting rates of arthropod vectors, enhancing the parasitism risk in wet areas in comparison with arid (Nunn *et al.* 2005) or marine (Mendes *et al.* 2005) environments.

The prevalence of *Leucocytozoon ziemanni* was positively correlated with forest cover around the nest (Table 2). Tella *et al.* (1999) also found that raptors inhabiting forested habitats showed higher prevalence of blood parasites and this result was linked with the low density of vectors in open areas. The

presence of wet depressions in vegetation and water accumulated in tree-holes can favour vector development (Garvin & Greiner 2003) and this might explain the higher prevalence of blood parasites in forests and their low incidence in bird species occupying arid and open lands (Little & Earlé 1995, Tella *et al.* 1999).

The positive relationship between habitat diversity and WBC concentrations (Table 1) is consistent with the prediction that living in more diverse habitats increases the chance of encountering vectors, reservoirs and parasites from other host taxa or environments (Gregory 1990, Nunn *et al.* 2003a, Vitone *et al.* 2004). In addition, it may be that inhabiting heterogeneous habitats could lead to increased dietary diversity in species with generalist food habits, increasing the opportunity for trophic disease transmission (Marcogliese 2004). A previous study in the Mediterranean region showed that Eagle Owls in more heterogeneous habitats had higher species richness in their diet (Penteriani *et al.* 2002). Further studies involving the diet composition of different breeding pairs are needed to test this hypothesis. Habitat diversity around the nest positively influenced the prevalence of blood parasites (Table 2). Greater habitat diversity may increase the contact with a higher number of species which can act as reservoirs for generalist pathogens (i.e. shared with other host species, e.g. Nunn *et al.* 2004). *Leucocytozoon ziemanni* infects a wide variety of owls (Remple 2004). Heterogeneous habitats could favour the presence of sympatric territories of different owl species (up to five species in the study area; Purroy 1997), leading to a higher host effective number and increasing the chance of being infected (Nunn *et al.* 2004). Furthermore, heterogeneous habitats may increase the risk of acquiring vector-borne diseases by means of facilitating the presence of a wide variety of oviposition and larval development sites for arthropod vectors (Garvin & Greiner 2003).

A high population density is likely to increase the horizontal disease transmission rate between hosts, favour re-infection processes and increase the chance of being infected with different genetic strains of pathogens, factors that are expected to enhance parasite virulence (Anderson & May 1979, Møller *et al.* 2001, Tella 2002). Thus, an elevated contact among hosts because of high densities usually leads to higher prevalence and diversity of parasites when density is analysed both at intraspecific (Côte & Poulin 1995) and at interspecific levels (Arneberg *et al.* 1998, Arneberg 2001, Tella 2002, Nunn *et al.* 2003a).

Accordingly, host population density was positively associated with the prevalence of blood parasites (Table 2). *Leucocytozoon ziemanni* is transmitted by haematophagous black flies, which acquire parasite gametocytes by biting an infected host. Hence, high host population density increases the chance of disease transmission (Tella 2002). Furthermore, distance between infected and susceptible hosts could be very important for blood parasite transmission, as vectors are known to suffer greater mortality when they acquire avian haemoparasites, limiting their dispersal ability (Valkiunas & Iezhova 2004).

The number of parasite species present increased with laying date, as did the prevalence of carnid flies and, weakly, the prevalence of blood parasites (Tables 2 & 3), suggesting that parasite populations increase as the reproductive season advances (reviewed in Merino *et al.* 2000). However, in spite of the higher parasitism pressure in broods from later breeding pairs, we found no association between WBC concentrations and laying date (Table 1). This could be due to a reduction in immunocompetence as breeding advances, which, in turn, is associated with a reduction in resources later in the season (Sorci *et al.* 1997, Dubiec & Cichon 2001; see, however, Merino *et al.* 2000).

Host condition, immune function and parasitism

We found no correlation between chick condition and WBC concentrations (Table 1). Several studies have found a negative association between condition and immunity (Møller *et al.* 1998), but others have not (Dubiec *et al.* 2005) or have found contradictory results depending on the year of study (Jovani *et al.* 2004) or the immune system parameter taken (Møller & Petrie 2002). Such differences could depend on whether the studied immune parameter reflects the healthy or disease status of an individual (Davis *et al.* 2004). By contrast, we did not observe any effect of chick condition on prevalence or number of parasite species. Several studies have reported a negative association between condition and parasitism (Møller *et al.* 1998, Davis *et al.* 2004) whereas others have found none (Allander & Sundberg 1997, Martínez-Abraín *et al.* 2002). This suggests that the transmission environment (vector abundance, habitats for parasite development, reservoirs, etc.) is a better predictor of disease risk than intrinsic parameters relating to host condition (Sol *et al.* 2000, Martínez-Abraín *et al.* 2002, Mendes *et al.* 2005).

Contrary to prediction, we found no significant effects of the prevalence of the studied parasites on WBC concentrations. The studied parasites have been considered as scarcely pathogenic and, thus, the immune system response against them could be weak (Adamo 2004). However, we only analysed a subset of potential parasites that the birds could have been carrying. Thus, nests free of the three parasites studied could be suffering other species of parasites not recorded in this study, masking any possible association between WBC concentrations and prevalence of the studied parasites. One strength of using immune system measures to assess disease risk is that they can capture the effects of the whole suite of parasites and pathogens, rather than a partial selection of them (Nunn 2002). But this also suggests that WBC concentrations may only show weak relationships with the three pathogens measured in the present study. The relationship between host immune system and disease clearly needs further research, especially concerning variation in the distribution of immune resources according to different types of parasites (Adamo 2004, Davis *et al.* 2004). Most comparative studies have analysed correlates of disease risk and immune system measures (e.g. Møller 1998, Møller *et al.* 2001, Nunn 2002, Semple *et al.* 2002, Nunn *et al.* 2003b) or parasite species prevalence (e.g. Nunn *et al.* 2003a, Vitone *et al.* 2004). However, the association between these two types of parameters has been rarely investigated (e.g. Morand & Poulin 2000). Such insight would allow us to assess directly whether the evolution of the immune system is linked to a higher risk of parasitism and would provide information about which parasites have exerted higher evolutionary pressures (Morand & Poulin 2000).

Implications for conservation research

Changes in land use and habitat destruction have been proposed as important factors affecting disease outbreaks (Daszak *et al.* 2000). Our results point to the importance of freshwater availability on the risk of parasitism via increasing vector abundance or parasite populations (see also Semple *et al.* 2002, Mendes *et al.* 2005, Nunn *et al.* 2005), a fact which is likely to play an important role in disease spread as a consequence of alterations of land use and human-induced climatic change (Harwell *et al.* 2002). Habitat destruction leads to shifts in species distribution toward less suitable habitats, where the chances of suffering diseases usually increases (Deem *et al.* 2001).

For instance, both habitat destruction and outbreaks of prey populations have led Eagle Owls to occupy areas linked with watercourses, where human pressure is lower and good prey populations survive (Ortego & Díaz 2004) but where we show they may suffer more from parasites. By contrast, habitat diversification, mainly originated by anthropogenic fragmentation of original habitats, can favour the proliferation of diseases (Allan *et al.* 2003) and increase the contact between organisms surviving in undisturbed habitats and other host taxa living in the disturbed habitats, facilitating a cross-species transmission of pathogens (Dobson & Foufopoulos 2001). Promoting habitat heterogeneity, which is known to be favourable for certain trophic and reproductive aspects in birds of prey (Sergio & Bogliani 2000, Penteriani *et al.* 2002, 2004), may lead to increased risk of acquiring parasites. Furthermore, high population densities in optimal habitats (Sergio & Newton 2003) may increase disease transmission. Protecting isolated subpopulations might prevent rapid disease spread that might occur if only high-density, continuous populations are preserved (Deem *et al.* 2001). Understanding the processes which govern parasite occurrence in natural populations is of great relevance to establishing management strategies that could allow us to focus efforts on protecting healthy populations and preventing epidemics that could lead to population declines (Daszak *et al.* 2000).

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