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DO HAEMOSPORIDIANS AFFECT FUEL DEPOSITION RATE AND FUEL LOAD IN MIGRATORY BLACKCAPS *SYLVIA ATRICAPILLA*?

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SUMMARY.—Do haemosporidians affect fuel deposition rate and fuel load in migratory blackcaps *Sylvia atricapilla*?

Aims: Fuel deposition rate is one of the main parameters determining bird migration strategies. Accordingly, factors compromising fuel deposition rate, such as parasite infections, might have relevant effects not only on migration, but also on other life history events that depend on migration success, such as breeding. We analysed the effect of haemosporidians on fuel load and fuel deposition rate in a population of migratory blackcaps (*Sylvia atricapilla*) during stopover in northern Iberia.

Locality: Loza lagoon, northern Iberia (42º 50' N, 01º 43' W, 400 m a.s.l.).

Methods: From blood samples of recaptured blackcaps we determined haemospiridian content by amplification of 479 bp of the parasite's cytochrome *b* gene. Independent on body size, of the sampled birds, 35 % were infected by *Haemoproteus-Plasmodium*.

Results: Mass deposition rate, fat score and body mass showed similar values in non-infected as in infected blackcaps. No differences in age or body size proportions were detected between infected and non-infected birds.

Conclusions: These results may be explained by infected birds with high virulence (compromising fuel accumulation) being unable to migrate south from their breeding areas in central and northern Europe. In contrast, more resistant birds may be able to tolerate the parasitaemia and gain fuel normally. Another possibility is that only birds with a low intensity of parasite infection were captured (presumably birds able to overcome the parasitaemia successfully), since these birds may be able to accumulate fuel at a similar rate to non-infected birds. Furthermore, highly infected birds may have a lower likelihood of capture than non-infected birds, because birds lose mobility with a high intensity of infection and under these circumstances they are less likely to be captured.

Key words: blackcap (*Sylvia atricapilla*), fuel load, fuel deposition rate, haemosporidians, migration, parasites.

RESUMEN.—Efecto de los hemosporidios en la carga de grasa y tasa de deposición de las currucas capirotadas migratorias (*Sylvia atricapilla*).

Objetivos: Uno de los parámetros que determinan la estrategia migratoria de un ave es la tasa de deposición de reservas. Así, cabe pensar que aquellos factores que influyan en esta variable podrían jugar

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un papel clave no sólo en la migración, sino en el resto de procesos del ciclo vital que dependan del éxito de la migración, como la reproducción. Se analizó hasta qué punto los hemosporidios afectaron la carga de grasa y su tasa de deposición en currucas capirotadas migratorias en un área de descanso en el norte de España.

Localidad: Laguna de Loza, norte de España (42º 50’ N, 01º 43’ W, 400 m s.n.m.).

Métodos: Se recogieron muestras de sangre en recapturas de currucas para, posteriormente, determinar la presencia de hemosporidios mediante la amplificación de un sector de 479 pares de bases de su citocromo *b.* En conjunto, se obtuvo una prevalencia del 35 % por *Haemoproteus-Plasmodium.* La tasa de deposición de peso, la carga de grasa y la masa corporal tuvieron un valor similar entre las aves con y sin infección.

Conclusiones: Es probable que los individuos en los que la virulencia pudo haber sido mayor no hubieran sido capaces de alcanzar el norte de España desde sus áreas de cría en el centro o norte de Europa. Contrariamente, las aves más resistentes habrían sido capaces de superar la parasitosis y de acumular reservas normalmente. Otra alternativa es que las aves que se capturaron tuvieran bajas tasas de infección, no teniendo en este caso los parásitos ningún efecto en la carga de grasa y su tasa de deposición. Finalmente, si en la zona de estudio hubo aves con altas tasas de infección (en las que el efecto sobre la carga de grasa podría haber sido evidente), es probable que no se capturaran ya que en estos casos la movilidad se ve seriamente reducida.

Palabras clave: curruca capirotada (*Sylvia atricapilla*), carga de grasa, tasa de deposición de grasas, haemosporidios, migración, parásitos.

INTRODUCTION

Parasites are one of the main factors compromising survival and breeding performance in their hosts, and hence determining their life histories. Most studies concerning parasitaemia have focused on parasite-mediated sexual selection, host-parasite co-speciation and evolution of parasite virulence (reviewed by Clayton and Moore, 1997). The effect of parasites on other life history aspects, such as migratory behaviour, has received relatively little attention (e.g. Valkiūnas, 1993; Dolnik, 2003; Zinke *et al.*, 2004).

Bird migration is usually divided into stopover, where migrants gain fuel, and flight phases, where distance is covered and energy consumed (Alerstam, 1990). Departure fuel load and rate of accumulation are key factors that reflect and determine avian migration strategies (Alerstam and Lindström, 1990; Hedenström and Alerstam, 1997). Birds able to gain fuel at faster rates minimise their stopover duration, are thus able to reach their goal areas in shorter time periods. Individuals arriving early will have advantages compared to those arriving later, with a higher territory quality (Francis and Cooke, 1986; Kokko, 1999). Accordingly, factors compromising fuel deposition rate, such as parasite infections, might have a relevant effect not only for migration, but also for other life history events that depend on mortality rates of migration, such as breeding success (Newton, 2004).

Haemosporidians are malaria and malaria-related apicomplexan protozoans which infect their hosts through insect vectors, usually diptera belonging to certain mosquito species, biting midges and blood-sucking flies (Valkiūnas, 2005). Genus *Plasmodium*, *Haemoproteus* and *Leucocytozoon* are common in many bird species and are reported to reduce fitness (e.g. breeding success) and cause morbidity and mortality (Clayton and Moore, 1997; Valkiūnas, 2005). Accordingly, at a given stopover locality, infected migrants probably show lower fuel deposition rates than non-infected ones, provided that haemoparasites sequester part of the hosts’ energy cycle, e.g. fuel accumulation (Peirce, 1984; Atkinson *et al.*, 1988). Conversely, fuel deposition rates could be similar between infected and non-infected migrants (Bennett *et al.*, 1988), especially if they have a low intensity of infection insufficient to compromise the fuel deposition rate of host (Bennett *et al.*, 1988; Valkiūnas, 2005).

The blackcap (*Sylvia atricapilla*) is a widespread western Palaearctic songbird, breeding from Iberia to western Russia, and from Scandinavia to northern Africa and the Macaronesian archipelagos (Shirihai *et al.*, 2001). Within the blackcap's migration system, there is a clear migratory divide around longitude 11º - 12ºE, with populations breeding west of the divide migrating in southwesterly directions, and those breeding east in southeasterly directions (Shirihai *et al.*, 2001). Accordingly, Iberia receives west European blackcap populations (mainly from France to southwestern Scandinavia, and from the British Islands to Germany; Cantos 1995), that either winter in, or travel through Iberia on their route to wintering areas in Africa (Cantos, 1995; Shirihai *et al.*, 2001).

We analysed the effect of haemosporidians on fuel load and fuel deposition rate in a population of migratory blackcaps at a stopover site in northern Iberia. We used a qualitative method to determine parasitaemia and to analyse whether fuel load or rate of fuel gain were lower in infected than in non-infected birds.

MATHERIAL AND METHODS

Sampling area and ringing protocol

Data were collected at the Loza lagoon, northern Iberia (42º 50’ N, 01º 43’ W, 400 m a.s.l.), approx. 40 km south of the Pyrenees. In a 50 ha site with meadows (75 - 80 % of the surface), reed beds (10 %) and hedgerows and poplar groves (10 - 15 %). Surrounded by urban areas and cereal fields, Loza is currently an oasis for migrating birds on stopover. Blackcaps were captured with mist nets placed across a 420 m long hedgerow line during 4 hours from dawn in 13 sampling days from 15 September to 7 October. Because of low recapture rates (Arizaga *et al.*, 2008), a tape lure was used in order to obtain a larger sample size.

Once captured, each blackcap was ringed, aged and sexed following Shirihai *et al.* (2001). We measured wing length, body mass, fat score and moult stage following Svensson (1998) and Kaiser (1993). Blood samples were taken from recaptured birds’ jugular vein (0.05 ml or less). This sample was stored in a FTA® card up to its use in the laboratory.

Determination of haemosporidian prevalence

We extracted DNA from blood kept in FTA® cards, following Gutiérrez-Corchero *et al.* (2002) and used a highly efficient nested Polymerase Chain Reaction (PCR) method (Waldenström *et al.*, 2004) to detect haemosporidians of genus *Haemoproteus*, *Plasmodium* and *Leucocytozoon* by amplification of 479 bp of the parasite's cytochrome *b* gene (Bensch *et al.*, 2000; Hellgren *et al.*, 2004; Waldenström *et al.*, 2004). All negative results were confirmed by repeating the PCR.

Mass deposition rate, fat load and body mass

We examined the variation of mass deposition rate, fat score and body mass independent from body size between infected and non-infected birds (see below for further details). We assessed mass deposition rate by subtracting the blackcaps’ body mass at recapture from body mass at first capture, divided by the number of days elapsed between these two dates. Blackcaps in our study area were found to gain mass at a constant rate (Arizaga *et al.*, 2008), thus, trapping on a daily basis was not crucial to accurate estimation of mass gain.

Fat score and body mass have different physiological significances. The first one represents the amount of fuel stored as subcutaneous fat in the ventral region (Kaiser, 1993), and it is correlated with the amount of body fat load. Body mass, by contrast, represents the general nutritional reserve, which also includes proteins, fat stores in other body parts and the relative size of organs.

Blackcaps normally stopover < 15 days at Loza (Arizaga *et al.*, 2008), thus, birds recaptured after more than 15 days were excluded from the analyses. Moulting birds were also omitted from the analysis, since they would be expected to accumulate fat in a different way to non-moulting migrants (Jenni and Winkler, 1994; Schaub and Jenni, 2001).

Statistics

We conducted *t* tests for mass deposition rate and fat score, with prevalence (infected and non-infected) as a factor. To test for differences in body mass independent of body size we conducted Univariate Linear Models (ULM) with body mass, wing length (used to assess body size, according to Gosler *et al.*, 1998) as covariate and prevalence as factor. The three dependent variables (mass deposition rate, fat score and body mass) fitted the normal distribution (K - S test, $P > 0.05$). SPSS (15.0) was used for statistics and we present all results with standard error (± SE).

RESULTS

Overall, 926 blackcaps were ringed during the study period. After removing moulting birds, and non-moulting birds recaptured after > 15 days, 53 valid recaptures were obtained. Among them, 18 (34.0 %) were infected with *Haemoproteus-Plasmodium* complex, whilst none were infected with *Leucocytozoon*.

Mass deposition rate had similar values between non-infected (-0.13 ± 0.11 g/day, $N = 35$) and infected individuals (-0.11 ± 0.13 g/day, $N = 18$; $t_{51} = 0.11$, $P = 0.91$). In a previous analysis, we found that birds recaptured after one day tended to have lower mass (Arizaga *et al.*, 2008), which could be associated with an energy-cost of settlement at the stopover site. In this study, 43 % of the birds were found to be recaptured after one day. Removing these birds from the analysis did not change the results ($t_{28} = 0.67$, $P = 0.51$), giving a mean mass deposition rate of 0.03 ± 0.07 g/day ($N = 30$).

Fat scores tended to be higher in non-infected (2.0 ± 0.2, $N = 35$) than among infected birds (1.7 ± 0.3, $N = 18$), however, this difference was not significant ($t_{51} = 0.99$, $P = 0.33$). The results did not change if removing birds recaptured after one day ($t_{28} = 1.18$, $P = 0.25$).

Body mass was also found to be similar between non-infected (18.3 ± 0.3, $N = 35$) and infected blackcaps (18.3 ± 0.3, $N = 18$; prevalence: $F_{1,52} = 0.01$, $P = 0.93$; wing length: $F_{1,52} = 0.04$, $P = 0.85$). Similar results were found if birds recaptured after one day were removed from the test (prevalence: $F_{1,28} = 0.15$, $P = 0.70$).

The proportion of age classes was similar between infected and non-infected birds (= 2.23, P-exact = 0.287), with 92.5 % juveniles. Similarly, mean wing length did not vary between infected and non-infected birds ($t_{51} = 0.421$, $P = 0.676$).

DISCUSSION

In our sample of migratory blackcaps 35 % were infected by *Haemoproteus-Plasmodium*. Using the same method, Pérez-Tris and Bensch (2005) reported a mean prevalence of almost 90 % for six different European blackcap populations. However, in a sample caught during winter in southern Iberia (Pérez-Tris and Bensch, 2005), 40 % were infected in a sample including both sedentary (local) and migrant (wintering) blackcaps (Pérez-Tris and Tellería, 2002). Hence, prevalence of haemosporidians in migratory blackcaps in northern Iberia reached a similar value as found during winter, and was much lower than observed during the breeding period. These differences between breeding and non-breeding periods is most probably due to the fact that parasitaemia peak during the Holarctic summer (Valkiūnas, 2005), whilst during autumn and winter the infection become chronic or latent, with gametocytes rarely found in blood.

Mass deposition rate, fat score and body mass independent from body size showed similar values in non-infected and infected blackcaps (for similar results in passerines see Bennett *et al.*, 1988). No differences in the proportion of age classes or body size (assessed with wing length, following Gosler *et al.*, 1998) were detected between infected and non-infected birds. Hence, three hypotheses could explain these results: (i) the haemosporidians had no effect on mass deposition rate and fuel load; (ii) the intensity of parasitaemia was not high enough to affect mass deposition rate and fuel load or (iii) the sample size was not large enough to detect differences.

Within a host population, resistant birds could show higher or more effective immune responses, or could change their behaviour in order to avoid or reduce the infection by parasites (reviewed in Clayton and Moore, 1997). In contrast, birds unable to give an adequate immune response could show higher mortality rates or be seriously affected by parasite infections (Clayton and Moore, 1997). The migratory blackcaps in our study were captured at a stopover site after a travel distance of hundreds to > 1,000 km from the breeding areas (Cantos, 1995; Fransson, 1995). Hence, it is possible that highly infected birds unable to fuel at a normal or expected rate, failed to migrate; therefore most of these birds did not reach northern Iberia. In contrast, more resistant birds presumably are able to tolerate the parasitaemia and hence will also be able to gain fuel normally, or at a rate high enough to complete their migration. To what extent the effect of an infection by haemosporidians could change along a given migratory route is still a question to be investigated.

Another possibility is that we only caught birds with low intensity of parasites (which could have overcome the infection successfully), which were able to fuel at similar rates to non-infected birds (Valkiūnas, 1991, 1993, 2005). Accordingly, in a sample of blood smears from migratory blackcaps collected at the study site in autumn, we found a mean rate of infection of 3 gametocytes/2,000 erythrocytes (Arizaga *et al.*, in prep.), a much lower value than that considered as an intense infection (more than 40 gametocytes/2,000 erythrocytes; Valkiūnas 2005).

Highly infected birds might have a lower capture rate than non-infected birds, because high intensity infections decreases mobility (Valkiūnas, 2005) and thus are less likely to be tangled in mist nets. The use of a tape lure could also have decreased capture rates of birds with high intensity infections (dominance effects). However, in blood smears from blackcaps caught without the use of a tape lure, we did not find high intensity infections (Arizaga *et al.*, in prep). Moreover, several studies support the hypothesis that tape lures attract presumably subdominant birds with lower fuel loads (Weatherhead and Greenwood, 1981; Greenwood *et al.*, 1986; Borrás and Senar, 1986). This might be the reason why registered mass deposition rates were low overall when compared with birds caught without the tape lure (Arizaga *et al.*, 2008).

The obtained mass deposition rates did not allow us to estimate the food intake rates, because we had periods of at least 24 h before recapturing the birds. During this time interval, food is expected to be metabolized, thus, we measured the net mass gain excluding energy used for metabolism (or by parasites). However, it is unlikely that infected birds would have higher intake rates than non-infected birds, since parasites normally cause morbidity and decreased activity (Valkiūnas, 2005).

In conclusion, even though our sample size was small, we believe the results are representative for migrating blackcaps. Bennett *et al.* (1988) did not find any effect of haemoparasites on body mass, even with a sample of > 3,500 individuals. As we have pointed out earlier, it is more likely that the lack of fuel load and deposition rate response to haemoparasite infections are associated with the intensity of infection among hosts, rather than with a sample-size biased effect (Bennett *et al.*, 1988; Valkiūnas, 2005).

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