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1 RH: DUNN ET AL. – BLOOD PARASITE INFECTION OVER WINTER  
2 **ACTIVE BLOOD PARASITE INFECTION IS NOT LIMITED TO**  
3 **THE BREEDING SEASON IN A DECLINING FARMLAND BIRD**

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9 ABSTRACT: Avian blood parasites can have significant impacts on adult breeding  
10 birds, but studies of parasitism outside the breeding season are rare, despite their  
11 potentially important implications for host-parasite dynamics. Here we investigate  
12 temporal dynamics of blood parasite infection in adult yellowhammers *Emberiza*  
13 *citrinella*. We screened blood samples collected between December and April of two  
14 consecutive winters using PCR. We found a high prevalence of both *Haemoproteus*  
15 and *Leucocytozoon* parasites, with a mean prevalence of 50% across 2 winters.  
16 Prevalence of both parasites was higher during the second, colder, winter of the  
17 study. Temporal trends differed between the 2 genera, suggesting that chronic  
18 *Haemoproteus* infections gradually disappear throughout the winter, but that  
19 *Leucocytozoon* infections exhibit a relapse during late winter, possibly coincident  
20 with reduced food availability. Our results highlight the difference in temporal  
21 dynamics between 2 blood parasite genera infecting the same host population and  
22 emphasise the need for accurate assessment of infection status at appropriate time  
23 periods when examining impacts of, and associations with, blood parasite infection.  
24 We suggest that further research should investigate the implications of over winter  
25 infection for birds' physiology, behaviour and survival.

26           Blood parasites can have a pronounced effect on reproduction in many bird  
27 species (Merino et al., 2000; Tomás et al., 2007; Knowles et al., 2009, 2010) and are  
28 associated with reduced survival in both naïve (Warner, 1968) and co-evolved hosts  
29 (Martínez-de la Puente et al., 2010; van Oers et al., 2010). Avian blood parasite  
30 infection can be associated with behavioural traits such as increased exploratory  
31 behaviour (Dunn et al., 2011) and morphology in terms of feather length (Rätti et al.,  
32 1993), and can also have ecological associations with later arrival date for migratory  
33 species (Rätti et al., 1993) and reduced survival (Martínez-de la Puente et al., 2010;  
34 van Oers et al., 2010). Despite the wide ranging effects of haemoparasites during the  
35 breeding season, such as reduced hatching, nestling provisioning and fledging  
36 success (Merino et al., 2000; Tomás et al., 2007; Knowles et al., 2010), the potential  
37 for them to affect hosts during the inter-breeding period has seldom been investigated  
38 (Allander and Sundberg, 1997). Environmental stressors may amplify impacts of  
39 parasite infection (Clinchy et al., 2004; Sih et al., 2004). Thus, during winter, when  
40 environmental stress in temperate climates can to be high due to low temperatures,  
41 increased flocking behaviour and a high requirement for scarce food resources,  
42 parasites may exert an additional pressure on populations (Barrow, 1963; Valkiūnas,  
43 2005).

44           Three separate genera of blood parasites commonly infect avian populations:  
45 *Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp. The 3 genera differ in  
46 their vectors and, to some extent, in their life cycles (Valkiūnas, 2005), but all 3 have  
47 been associated with detrimental impacts on their hosts (e.g. *Plasmodium*: Van Riper  
48 et al., 1986; *Leucocytozoon*: Bunbury et al., 2007; *Haemoproteus*: van Oers et al.,  
49 2010).

50           The prevalence of patent blood parasite infection (when parasites can be  
51 detected circulating in the blood, rather than dormant in tissues) varies temporally  
52 (Bensch and Åkesson, 2003; Cosgrove et al., 2008), and the prevalence of all 3  
53 genera tends to peak at the beginning of the breeding season in temperate climates  
54 (Sundberg, 1995; Allander and Sundberg, 1997; Valkiūnas, 2005; Cosgrove et al.,  
55 2008). This peak is due to relapse of existing infections as the onset of breeding  
56 leads to rising levels of hormones such as corticosterone (Applegate, 1970; Valkiūnas  
57 et al., 2004). In tropical and subtropical climates, patent parasite infection can be  
58 found throughout the year because transmission can occur continuously; however, in  
59 temperate climates transmission tends to cease outside the breeding season as  
60 temperatures fall and vector activity ceases (Cosgrove et al., 2008). Plasmodium  
61 infections tend to clear from the blood completely outside the non-breeding season  
62 (Applegate, 1970; Cosgrove et al., 2008). However, gametocytes from chronic  
63 Haemoproteus and Leucocytozoon infections can remain in the blood for many  
64 months after initial infection (Valkiūnas, 2005) but relatively little is known about the  
65 period for which parasites can be detected in the blood, or the impact that chronic  
66 infections may have on host ecology outside the season of active parasite  
67 transmission.

68           Factors outside the breeding season can also cause increased corticosterone  
69 levels and may potentially induce relapses of existing parasite infections (Barrow,  
70 1963; Applegate, 1970; Valkiūnas, 2005). Extreme weather conditions (Romera et  
71 al., 2000), food restriction (Kitaysky et al., 2001) and poor habitat quality (Marra and  
72 Holberton, 1998) can all increase corticosterone levels and these effects may occur at  
73 any time of year, suggesting potential interactions with the dynamics of  
74 haemoparasite infections (Valkiūnas et al., 2004). Thus, in species where other

75 stress-inducing factors, such as food shortages or habitat degradation, act outside the  
76 breeding season, a stress-induced decrease in immunity might either trigger parasite  
77 relapse or cause a delay in clearing parasites from the bloodstream. Multiple stress-  
78 inducing factors can have synergistic effects (Clinchy et al., 2004; Sih et al., 2004),  
79 both physiologically (Clinchy et al., 2004) and with ecological consequences (Zanette  
80 et al., 2003); thus, patent parasite infection may exacerbate the effects of food or  
81 weather related stress. Levels of parasite infection might thus be higher during  
82 periods of increased stress, such as during colder winters, than during milder winters.

83 Here, we investigate the temporal dynamics of blood parasite prevalence  
84 during the non-breeding season in a population of Yellowhammers *Emberiza*  
85 *citrinella*, a farmland bird whose downward population trend (Eaton et al., 2011) has  
86 been associated with decreased over winter survival (Bradbury et al., 2000). We  
87 sampled our population over 2 winters varying markedly in temperature, and we  
88 describe the temporal variability of patent infection across our population.

## 89 **MATERIALS AND METHODS**

### 90 **Study population and blood sampling**

91 Work was carried out within an individually marked population of  
92 Yellowhammers near Tadcaster, North Yorkshire (53°53'N, 1°15'W). Birds were  
93 caught in static mist nets and whoosh nets (Redfern and Clark, 2001) at an  
94 established supplementary feeding site baited sporadically with wheat and weed  
95 seeds, within an experimental agroforestry block surrounded by arable farmland.  
96 Two hundred and three birds were caught on 30 sampling occasions between  
97 November 2007 and April 2009. Nineteen birds were caught and sampled on 2  
98 occasions within this period and 3 birds were caught and sampled on 3 separate  
99 occasions more than 2 mo apart.

100 Birds were aged and sexed by plumage (Svensson, 1992; Dunn and Wright,  
101 2009). Blood was taken through venipuncture of the brachial vein and stored with  
102 EDTA as an anticoagulant prior to freezing.

### 103 **DNA extraction and detection of blood parasites**

104 DNA was extracted from 30  $\mu$ l of whole blood using a standard phenol-  
105 chloroform extraction followed by ethanol precipitation (Sambrook et al., 1989).  
106 Successful DNA extraction was confirmed by using a Nanodrop ND-1000  
107 Spectrophotometer (Nanodrop Technologies Inc., Wilmington, Delaware) and  
108 extracted DNA was diluted to a working concentration of 25 – 100 ng/ $\mu$ l.

109 Blood parasite presence or absence was determined through PCR using 2  
110 established protocols. The presence of Plasmodium and Haemoproteus was  
111 established using primers HaemF and HaemR2 nested within HaemNF and  
112 HaemNR2 (Waldenström et al., 2004), and Leucocytozoon spp. were detected using  
113 primers HaemFL and HaemR2L nested within primers HaemNFI and HaemNR3  
114 (Hellgren et al., 2004). All protocols were carried out in a working volume of 25  $\mu$ l  
115 containing 50 – 200 ng template DNA, 1.25 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 0.4  $\mu$ M of  
116 each primer, 1 x GoTaq Flexi Buffer (Promega, Madison, Wisconsin) and 1 U GoTaq  
117 Flexi (Promega); a positive control of DNA from a bird with known infection and a  
118 negative control containing deionised water in place of DNA were included with each  
119 PCR reaction to ensure successful amplification, and lack of contamination,  
120 respectively.

121 PCR protocols were identical for detection of both parasite genera. First round  
122 reactions consisted of a denaturation step of 94 C for 3 min followed by 20 cycles of  
123 94 C for 30 sec, 50 C for 30 sec and 72 C for 45 sec, with a terminal extension step of  
124 72 C for 10 min; the protocol for second round reactions contained 35 cycles but

125 otherwise consisted of an identical thermal profile. PCR protocols were carried out  
126 on a GeneAmp PCR System 9700 (Applied Biosystems). Non-target DNA can be  
127 amplified with nested PCR methods (Szöllösi et al., 2008), so a subsample of 38  
128 positive samples from 34 birds were sequenced using an ABI sequencer at the Core  
129 Genomic Facility, Sheffield University (Sheffield, South Yorkshire, UK). Identity of  
130 parasites was confirmed by comparison with sequences in GenBank using the NCBI-  
131 BLAST database (Altschul et al., 1997).

132         Blood smears were created from a subset of positive samples (n=44) and  
133 examined under an oil immersion x 100 magnification lens. Haemoproteus infection  
134 intensity was assessed at the same time as white blood cell (WBC) differentials  
135 (results reported in Dunn et al., 2013) and thus was assessed from the number of  
136 microscope fields required to find 100 WBCs (mean  $\pm$  1 SE: 1242  $\pm$  150 microscope  
137 fields; 236927  $\pm$  29595 erythrocytes). We then assessed the number of intracellular  
138 parasites in non-distorted erythrocytes to establish Haemoproteus infection intensity  
139 and standardised this measure to reflect the number of parasites per 10,000  
140 erythrocytes. We did not assess Leucocytozoon infection intensity.

#### 141 **Statistical analyses**

142         All analyses were carried out in R version 3.0.2 for Mac (R Core  
143 Development Team, 2009). Where 2 or more data points existed from the same  
144 individual, 1 was selected at random and retained, and the rest deleted to avoid  
145 pseudoreplication. To ensure this retained sample was representative, we repeated  
146 this three times to ensure results were consistent between datasets. To examine  
147 factors influencing variation in parasitism, we used 2 general linear models (for each  
148 parasite genus separately) with binomial error structures and infection status as the  
149 response variable. The fixed factors we examined were year, day (as a continuous

150 variable where Nov 1 = 1, allowing for linear and quadratic relationships), age and  
151 sex, as well as two-way interactions between year and day to allow for the possibility  
152 of year-dependent relationships with day: we examined all possible candidate models  
153 using the ‘dredge’ function in the ‘MuMIn’ library (Barton, 2012) and ranked models  
154 using second-order Akaike’s Information Criteria (AICc). AICc measures the  
155 relative goodness of fit of a model and takes into account the number of variables  
156 within each model, penalising models for the addition of variables, thus selecting for  
157 a model with the maximum goodness of fit and retaining the minimum number of  
158 explanatory variables (Burnham and Anderson, 2002). Where more than 1 candidate  
159 model had  $\Delta AIC < 2$ , we averaged these models to provide parameter estimates  
160 adjusted for shrinkage according to the number of top models within which each term  
161 was found (Burnham and Anderson, 2002).

## 162 **RESULTS**

### 163 **Parasite prevalence and identity**

164 Two hundred and twenty-five blood samples from 203 birds were screened  
165 for the presence of *Plasmodium* spp. and *Haemoproteus* spp. Using the protocol of  
166 Waldenström et al. (2004), 105 of 225 samples (47 %) tested positive for  
167 *Plasmodium* spp. and *Haemoproteus* spp. A subset of 195 samples was selected at  
168 random and tested using the protocol of Hellgren et al. (2004) for detection of  
169 *Leucocytozoon* spp., and 52 of 195 samples (27 %) contained parasites. Of the 52  
170 birds testing positive for *Leucocytozoon* spp., only 7 were not infected by  
171 *Haemoproteus* spp. giving an overall parasite prevalence of 50 %. Co-infection by  
172 both *Haemoproteus* spp. and *Leucocytozoon* spp. occurred more frequently than  
173 expected by chance ( $\chi^2_1=44.0$ ,  $p<0.001$ ): 51 % of birds infected by *Haemoproteus*



174 spp. were also infected by *Leucocytozoon* spp., whereas 89% birds infected by  
175 *Leucocytozoon* spp. were also infected by *Haemoproteus* spp.

176 Thirty-eight parasite sequences were obtained from 34 infected birds. Of  
177 these, 1 sequence was identified as a novel *Leucocytozoon* lineage, designated  
178 EMCIT01 (Genbank accession number JQ346795), and the remainder were identified  
179 as *Haemoproteus* lineages DUNNO01 and EMRUT01 (Genbank accession numbers  
180 DQ991080 and EF380192). The *Leucocytozoon* lineage was amplified using  
181 Hellgren et al. (2004) and all the *Haemoproteus* lineages were amplified using  
182 Waldenström et al. (2004).

183 Median infection intensity of *Haemoproteus* spp. in a random subsample of  
184 birds (subsample selected based on the quality of the blood smear and blind to the  
185 brightness of the PCR band) confirmed as infected through PCR (n=44) was 0.38  
186 parasites per 10,000 erythrocytes (range 0 – 7.03 parasites per 10,000 erythrocytes).  
187 Parasites were detected in 24 of the 44 smears.

#### 188 **Associations with host and environmental variables**

189 Day and year strongly influenced the prevalence of both *Haemoproteus* and  
190 *Leucocytozoon*, being retained in all top models for each parasite genus (Table I).  
191 Confidence intervals for day (both linear and quadratic terms) and year did not  
192 overlap zero for either parasite genus (Table II), suggesting a strong, non-linear,  
193 influence of day on parasite prevalence, as well as a higher prevalence of both  
194 parasite genera during the second year of the study (Figures 1 & 2). *Haemoproteus*  
195 prevalence increased from 39% in 2007/08 to 66% in 2008/09, whereas  
196 *Leucocytozoon* prevalence increased from 20% in the winter of 2007/08 to 50% in  
197 2008/09. While both interaction terms were retained in each averaged final model,  
198 confidence intervals for all interaction terms spanned zero, suggesting no differences

199 in temporal variation in prevalence between years. During both years, *Haemoproteus*  
200 prevalence declined gradually throughout the winter, although predicted values  
201 suggest a slight increase towards the end of February (day 120; Figure 1).  
202 *Leucocytozoon* prevalence declined gradually until early-February (day 100) and then  
203 increased markedly (Figure 2). Predicted prevalence of either parasite did not  
204 approached zero in either year.

205 Host age was retained in only 2 of the 5 models examining *Haemoproteus*  
206 prevalence and 1 of the 7 models examining *Leucocytozoon* prevalence (Table I), and  
207 confidence intervals overlapped zero for both models (Table II). Similarly, host sex  
208 was retained in only 1 of the 5 models examining *Haemoproteus* prevalence, and 3 of  
209 the 7 models examining *Leucocytozoon* prevalence (Table I), and confidence  
210 intervals overlapped zero for both models (Table II), so neither variable is considered  
211 further.

## 212 **DISCUSSION**

213 We found an overall parasite prevalence of 50 % in our population during the  
214 non-breeding season over 2 yr, which was, on average, low compared to a previous  
215 prevalence of 70 % in breeding yellowhammers (Sundberg, 1995). However,  
216 *Haemoproteus* prevalence approached 70 % overall during 2008. Typically,  
217 haemoparasite infections relapse at the beginning of the breeding season, when  
218 circulating corticosterone levels increase (Sundberg, 1995; Cosgrove et al., 2008).  
219 Outside the breeding season in temperate environments, *Plasmodium* infections tend  
220 to disappear from circulating blood rapidly, with over winter prevalence at 0 %  
221 (Cosgrove et al., 2008). Less is known about the seasonal dynamics of  
222 *Haemoproteus* and *Leucocytozoon* in passerine hosts, although a previous study of  
223 *Haemoproteus* in breeding yellowhammers found a pre-breeding relapse during late

224 April and throughout May, and suggested that infections may last beyond the  
225 breeding season albeit at low intensities, although to our knowledge this was not  
226 subsequently investigated (Sundberg, 1995; Allander and Sundberg, 1997). Previous  
227 studies of haemoparasites in other systems have found various prevalences of  
228 infection outside the breeding season: for example, Barnard and Bair (1986) found  
229 *Leucocytozoon* in 16.5 % of 50 bird species, mostly passerines, examined between  
230 December and March in Vermont, although no *Haemoproteus* infections were found  
231 between November and April despite the presence of suitable hosts; *Haemoproteus*  
232 prevalence peaked at ~33 % during September. Barnard et al. (2010) found a 42 %  
233 *Leucocytozoon* prevalence during both summer and winter in rusty blackbirds  
234 *Euphagus carolinus*, and found no *Haemoproteus* infections in breeding rusty  
235 blackbirds compared to infections in 3 % of wintering birds. Deviche et al. (2010)  
236 found an over winter dip in prevalence of *Leucocytozoon fringillinarum* infection in  
237 white-winged crossbills *Loxia leucoptera*, but found this parasite genus was absent  
238 from sampled birds for only a short period during January, suggesting that the early  
239 breeding season of the crossbill initiated a pre-breeding relapse in March and April.  
240 Conversely, for *Haemoproteus fringillae*, this study found parasites were absent from  
241 circulating blood between January and April, suggesting that a seasonal relapse in  
242 May was indicative of novel infections following an increase in vector activity  
243 (Deviche et al., 2010), a pattern also found by Schrader et al. (2003) in red-bellied  
244 woodpeckers *Melanerpes carolinus*, where *Haemoproteus* prevalence was 0 % in  
245 January and February but peaked at 80 % in July. All 4 of these studies used blood  
246 smears alone for detection of haemoparasite infections (Barnard and Bair, 1986;  
247 Schrader et al., 2003; Barnard et al., 2010; Deviche et al., 2010), suggesting that  
248 actual prevalence may be higher because blood smears can be relatively insensitive

249 compared to PCR for detecting low intensity infections: for example, Fallon and  
250 Ricklefs (2008) found 35 % of PCR-detected Haemoproteus infections to not be  
251 detected on blood smears. Although PCR cannot distinguish between infective and  
252 non-infective stages (Valkiūnas et al., 2011), Haemoproteus species only cast  
253 infective gametocytes in the bloodstream and are not found in the blood as non-  
254 infective asexual stages (Pérez-Tris and Bensch, 2005; Valkiūnas, 2005), and thus  
255 any detection of Haemoproteus in the bloodstream indicates an active infection.

256         Interestingly, co-infection by multiple parasites was not random, with birds  
257 infected by Leucocytozoon 23.1 times more likely to be infected by Haemoproteus  
258 than those not infected by Leucocytozoon. Initial infection may be correlated: whilst  
259 Haemoproteus and Leucocytozoon are transmitted by different vectors (from the  
260 families Ceratopogonidae and Hippoboscidae, and the family Simuliidae  
261 respectively; Valkiūnas, 2005), both vectors may be more abundant in wet areas (e.g.  
262 Wood et al., 2007). Individual behaviour may make individuals more likely to  
263 encounter vectors: for example, female great tits infected by haemoparasites were  
264 more exploratory than uninfected individuals, suggesting a behavioural pre-  
265 disposition to infection (Dunn et al., 2011).

266         Examination of temporal trends in infection prevalence in our data suggests  
267 different patterns for Haemoproteus and Leucocytozoon infections. Leucocytozoon  
268 infections show a gradual decline in prevalence until early-February, after which  
269 prevalence starts to increase markedly, unlike Haemoproteus infections which appear  
270 to increase slightly towards the end of February. This suggests that, for both genera,  
271 we were seeing a gradual decline in patent infections as parasites were cleared from  
272 the bloodstream, albeit over a lengthy period. This is supported by our infection  
273 intensity data for Haemoproteus that showed levels in our population to be relatively

274 low compared to other passerine-Haemoproteus spp. systems (e.g. Bensch et al.,  
275 2000: 40-790 parasites per 10,000 RBCs; Fallon and Ricklefs, 2008: means of 6.4  
276 and 12.1 parasites per 10,000 RBCs in the West Indies and Missouri Ozarks  
277 respectively; Asghar et al., 2011: 90 parasites per 10,000 RBCs) during the summer  
278 months. Unfortunately we cannot make direct comparisons with breeding season  
279 infection intensity in our study species because, to our knowledge, these data are only  
280 presented in Allander and Sundberg (1997) who measured parasites per 100  
281 microscope fields, and did not standardise measurements by erythrocyte abundance,  
282 and we did not sample individuals during the breeding season. Future work could  
283 test this by collecting more extensive infection intensity data and establishing  
284 whether a decline in infection intensity occurs concurrent with reduced prevalence,  
285 expected if the pattern we observe is due to declining chronic infections.

286         After early-February, *Leucocytozoon* infection prevalence increases markedly,  
287 and *Haemoproteus* infection prevalence increases slightly from the end of February.  
288 This suggests a relapse of existing infection rather than a decline in chronic infection.  
289 Transmission of all 3 blood parasite genera in temperate regions is thought to be  
290 negligible throughout the winter due to a cessation of vector activity (Cosgrove et al.,  
291 2008; but see also Klei and DeGiusti, 1975 for high over winter vector activity).  
292 However, parasites remain dormant in host tissues (Valkiūnas, 2005) and are  
293 activated by stress hormones, usually at the onset of breeding, when relapses occur  
294 and parasites can be found circulating in the blood (Applegate, 1971; Allander and  
295 Sundberg, 1997). Yellowhammers start breeding relatively late, with mean first egg  
296 date on 29 May and the earliest broods being initiated in early May (Bradbury et al.,  
297 2000). Our latest sampling point during both years was 23 April, and thus there is  
298 minimal overlap between our data collection and the onset of the breeding season,

299 suggesting that this increase in parasite prevalence in our population is independent  
300 of breeding-induced relapse. We suggest 3 possibilities for this apparent relapse of  
301 infection.

302         Firstly, host immunity can be lowered during the winter (Hasselquist et al.,  
303 1999; Møller et al., 2003; Hasselquist, 2007), which may allow a relapse of existing  
304 infections because reduced immune function is often associated with increased  
305 parasite prevalence (Ots and Hörak, 1998; Barnard et al., 2010). However, a multi-  
306 species study including yellowhammers provided little evidence for a reduction in  
307 winter immunity in this species, although the sample size was small (Møller et al.,  
308 2003).

309         The second possibility is that a reduction in over winter food availability may  
310 trigger a relapse of infection through increased circulating corticosterone levels:  
311 whilst our population was sampled at a supplementary feeding site, this was baited  
312 only sporadically and thus cannot be considered a reliable source of food. A  
313 reduction in over winter food availability has been linked to population declines in  
314 many farmland bird species, including yellowhammers (Peach et al., 1999; Robinson  
315 and Sutherland, 1999; Bradbury et al., 2000) and food availability in the wider  
316 countryside is thought to be insufficient from February onwards (Siriwardena et al.,  
317 2008). Corticosterone levels can increase at times of low food availability (Kitaysky  
318 et al., 2001; Clinchy et al., 2004) and poor weather (Romera et al., 2000), and  
319 increased corticosterone levels have been experimentally linked to both an increased  
320 parasite prevalence and an increased intensity of infection (Applegate, 1970). It  
321 seems plausible, and temporally relevant, that a reduced food supply may either  
322 induce relapses of haemoparasite infection or delay clearance of gametocytes from  
323 the host blood stream. This possibility is supported by the higher prevalence of both

324 parasites during the colder winter of 2008/09 than the relatively mild winter of  
325 2007/08. The potential implications of food-stress influencing the temporal dynamics  
326 of parasites, and subsequent implications for survival require further exploration.

327         A third explanation, although one that appears unlikely, is that the infections  
328 we observe result from active transmission of parasites resulting in novel infections.  
329 Little is known of the ecology of vectors in our population, so we cannot discount  
330 continuing vector transmission during the winter months; indeed, where vectors are  
331 present, over winter transmission has been recorded (Klei and DeGiusti, 1975).  
332 However, if this were the case then we would expect parasite prevalence to be higher  
333 during a warmer winter when more vectors would be likely to survive, whereas in  
334 fact we found prevalence to be higher during the colder winter of 2008/09, also  
335 coincidental with lower bird numbers despite similar sampling effort. This instead  
336 supports the idea of a higher incidence of stress-induced relapse in birds that survived  
337 the early cold spell during the autumn of 2008 (National Climate Information Centre,  
338 2008), which is likely to have caused high mortality resulting also in the lower  
339 number of birds caught during this winter.

340         The high prevalence of over winter infection found in our study population  
341 suggests a previously overlooked and potentially important role of haemoparasites  
342 during the non-breeding season, possibly initiated, for *Leucocytozoon*, by food stress  
343 in our population. The implications of this previously over-looked period of active  
344 blood parasite infection for both host and parasite life-histories and population  
345 dynamics need further investigation. Further understanding the dynamics of  
346 *Haemoproteus* and *Leucocytozoon* infection emphasises the importance of long-term  
347 studies of host-parasite systems that allow repeated sampling of individuals through  
348 time.

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524

525 FIGURE 1. The prevalence of Haemoproteus infection varied over time and between  
526 years. Points show raw data; lines are those predicted from the final models (Table  
527 I).

528

529 FIGURE 2. The prevalence of Leucocytozoon infection varied over time and  
530 between years. Points show raw data; lines are those predicted from the final models  
531 (Table I).

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