



**HAL**  
open science

## **Coccidial oocyst release: once a day or all day long? Tropical bird hosts shed new light on the adaptive significance of diurnal periodicity in parasite output**

Clotilde Biard, Karine Monceau, Maria Teixeira, Sébastien Motreuil, Soline Bettencourt-Amarante, Lucie Develay, Jérôme Moreau

### ► To cite this version:

Clotilde Biard, Karine Monceau, Maria Teixeira, Sébastien Motreuil, Soline Bettencourt-Amarante, et al.. Coccidial oocyst release: once a day or all day long? Tropical bird hosts shed new light on the adaptive significance of diurnal periodicity in parasite output. *Parasitology*, 2022, 149 (4), pp.469 - 481. 10.1017/S003118202100202X . hal-03461123

**HAL Id: hal-03461123**

**<https://hal.science/hal-03461123>**

Submitted on 24 Feb 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Coccidial oocyst release: once a day or all day long? Tropical bird hosts**  
2 **shed new light on the adaptive significance of diurnal periodicity in parasite**  
3 **output**

4

5 Clotilde Biard<sup>1</sup>, Karine Monceau<sup>2</sup>, Maria Teixeira<sup>3</sup>, Sébastien Motreuil<sup>3</sup>, Soline Bettencourt-  
6 Amarante<sup>3</sup>, Lucie Develay<sup>3</sup>, Jérôme Moreau<sup>2,3\*</sup>

7

8 <sup>1</sup>Sorbonne Université, UPEC, Paris 7, CNRS, INRA, IRD, Institut d'Écologie et des Sciences  
9 de l'Environnement de Paris, F-75005 Paris, France.

10 <sup>2</sup>Centre d'Études Biologiques de Chizé, UMR 7372, CNRS & La Rochelle Université, 79360  
11 Villiers-en-bois, France

12 <sup>3</sup>UMR CNRS 6282 Biogéosciences, Université Bourgogne Franche-Comté, 6 Boulevard  
13 Gabriel, 21000 Dijon, France

14

15 \* **Author for correspondence:** Jérôme Moreau, Email: [jerome.moreau@u-bourgogne.fr](mailto:jerome.moreau@u-bourgogne.fr)

16

17

18 **Abstract**

19 **Many parasites spend part of their life cycle as infectious forms released from an infected host**  
20 **in the external environment, where they may encounter and infect new hosts. The emergence**  
21 **of infectious life stages often occurs once a day to minimize mortality in adverse environments.**

22 In bird hosts, intestinal parasites like coccidia are generally released with faeces in the late  
23 afternoon. This dynamic is adaptive since it allows avoiding desiccation and UV radiations,  
24 thus reducing mortality of oocysts in the environment until transmission to the next host. If this  
25 circadian rhythm is the result of natural selection to increase oocyst survival, we may  
26 hypothesize that oocysts will appear in faeces at different times depending on the environment  
27 where hosts live. Particularly, in an environment where UV radiations and desiccation are very  
28 low, we may expect oocyst circadian release to disappear since the main selective pressure  
29 would be relaxed. We sampled different species of birds in tropical and temperate forests in  
30 spring and investigated coccidian oocyst output. A strong circadian variation in the prevalence  
31 of hosts shedding coccidian oocyst was detected for species caught in the temperate forest with  
32 an increase in prevalence in the late afternoon, whereas prevalence of birds shedding oocysts  
33 was constant over the course of the day for most species sampled in the tropical rain forest.  
34 These results are consistent with the hypothesis that oocysts circadian output is maintained by  
35 natural selection to increase oocyst survival. We discuss the adaptive significance of diurnal  
36 periodicity in parasite output.

37

38 **Key words:** circadian rhythms, Coccidia, temperate forest, tropical forest, host-parasite  
39 interactions, birds

40

41

42

### 43 **Key Findings**

- 44 • Coccidia in birds are released with faeces in late afternoon
- 45 • This circadian output disappears for tropical forest birds
- 46 • In this environment the sun radiation reaching the ground is dramatically reduced
- 47 • Survival of the oocysts in this external environment are then maximum
- 48 • Natural selection shape rhythms for *Isospora* sp. in passerine birds

### 49 **Introduction**

50 From an evolutionary perspective, natural selection should favour any trait increasing the  
51 probability of parasite transmission from one host to another (Bush *et al.*, 2001; Combes, 2001).  
52 For example, many parasite species with complex life cycles (i.e., parasites with successive  
53 host species), have evolved strategies of releasing infective forms at times of the day when the  
54 definitive host is most likely to be present in the environment (Théron, 1984; Combes *et al.*,  
55 1994; Combes, 2001 and references therein). Such a phenomenon of diurnal periodicity is also  
56 observed in parasites with simple life cycles (i.e., parasites with a single host species). Coccidia  
57 from the genus *Isospora* (Protozoa, Apicomplexa) are intracellular intestinal parasites of birds,  
58 and typically peak oocyst release in the late afternoon (Boughton, 1933; Kruszewicz, 1995;  
59 Brawner and Hill, 1999; Dolnik, 1999a, 1999b; Hudman *et al.*, 2000; Brown *et al.*, 2001; Misof,  
60 2004). These protozoans exhibit both sexual and asexual phases. In most species, one or more  
61 asexual multiplication's cycles are completed in the intestinal epithelium of the host, before  
62 sexual development occurs (Olsen, 1974). Zygotes produced during the sexual phase transform  
63 into oocysts that are then released into the host's intestinal tract and excreted within faeces. To  
64 complete their development, oocysts must sporulate outside the host to become infective before  
65 being ingested by their new host (see for an example Abd-Al-Aal *et al.*, 2000).

66           In birds, coccidian oocyst shedding shows a strong circadian pattern that appears to be  
67 ubiquitous since in all species tested to date (16 species), oocyst shedding has been found to  
68 peak in the afternoon (Knight *et al.*, 2018), whatever the latitude considered, from the tropics  
69 zone to arctic area (Dolnik *et al.*, 2011). Even in an extreme environment with permanent  
70 daylight like the arctic, this periodicity is conserved despite the absence of diurnal periodicity  
71 in hosts' activity (Dolnik *et al.*, 2011). The peak in oocyst shedding late in the afternoon is  
72 considered an adaptative trait providing resistance to desiccation and UV radiations for oocysts  
73 in the external environment (Martinaud *et al.*, 2009). Indeed, a single hour of exposure of the  
74 host's faeces to natural sunlight reduced the viability of oocysts by ca. 50%, which further  
75 decreased with continued exposure (Martinaud *et al.*, 2009). Therefore, releasing oocysts  
76 throughout the day would result in a large proportion quickly dying due to sunlight exposure,  
77 and a decrease in transmission probability to the next host. Interestingly, all bird species  
78 showing temporal variation in the shedding of coccidia oocysts with a peak late in the afternoon  
79 roughly have in common one characteristic of their environment: a ground exposed to strong  
80 solar radiations including the arctic area (Knight *et al.*, 2018). However, an exciting question  
81 that remains open is whether oocysts shedding in an environment where the sun reaching the  
82 ground is dramatically reduced throughout the year still shows diurnal periodicity. Indeed, in  
83 such environments, the selective pressure of desiccation and UV exposure of oocysts during  
84 the daytime would be relieved. One would therefore predict periodicity in oocyst shedding  
85 either not to evolve or be lost as it would reduce transmission probability without the benefits  
86 of increasing oocysts viability and should thus be selected against.

87           Although tropical rainforests receive 12 hours of sunlight daily, it is generally assumed  
88 that less than 2% of radiations ever reach the ground. Tropical rainforests have dense  
89 vegetation, often forming three different layers including a dense canopy that blocks out ca.  
90 95% of sunlight (Brinkman, 1971; Bourgeron, 1983; Chazdon and Fetcher, 1984; Vazquez-

91 Yanes *et al.*, 1990). Therefore, the rainforest ground rarely receives direct sunlight. Also,  
92 tropical rainforests are characterized by high air humidity, ranging on average from 80 to 90%  
93 relative humidity (RH; Freiberg, 1997; Bruno *et al.*, 2006) and soil moisture. By combining  
94 low direct sunlight and high RH, this environment provides faeces shed by birds with very  
95 favourable conditions to allow oocyst sporulation and survival. If temporal variation in  
96 shedding of coccidial oocysts is under natural selection to avoid desiccation and UV radiations  
97 as hypothesized, we may predict that no circadian rhythm should be observed in tropical forests  
98 contrary to temperate forests. Indeed, temperate forests are exposed to a stronger irradiance and  
99 lower soil moisture compared to tropical forests since the vegetation canopy is moderately  
100 dense and allows light to penetrate (Szwagrzyk *et al.*, 2001). However, to our knowledge,  
101 temporal variation in the shedding of coccidia oocysts has never been investigated in a tropical  
102 rain forest environment.

103         To investigate whether periodically synchronised release of coccidia oocysts from the  
104 genus *Isospora* may be absent in tropical forests, consistent with the hypothesis of relaxed  
105 natural selection linked to UV radiations and desiccation, we compared patterns of oocyst  
106 shedding from birds living in the primary rain forest in French Guyana (low luminosity and  
107 high humidity and moisture) and that from birds living in open temperate forests in mainland  
108 France (high ground luminosity and low humidity and moisture). The generality of this  
109 expected difference in patterns of oocyst shedding across the diversity in host ecology and life  
110 history was assessed by sampling a variety of bird species belonging to different families within  
111 both habitat types. We expected marked temporal variation in the shedding of coccidia oocysts  
112 for host species living in open forest and no or weak temporal variation for hosts living inside  
113 the dark rain forest. Besides, we took advantage of the spatial heterogeneity in vegetation cover  
114 existing around the field station located in the tropical rain forest to study variation in oocyst  
115 shedding patterns at a smaller scale. Indeed, a large area has been maintained open at the edge

116 of the field station for more than 30 years (Fig. 1) and has selected bird species specialists of  
117 open habitats. We, therefore, expected temporal variation in the shedding of coccidia oocysts  
118 from host species inhabiting this open area, contrary to those inhabiting the close by rain forest.

119

## 120 **Materials and methods**

### 121 *Trapping birds and sampling faeces*

122 Adult males and females of different bird species (one-year-old and older, identified by  
123 plumage characteristics) were caught using mist-nets from sunrise to sunset in both tropical and  
124 temperate areas. Species and age were determined according to handbooks (Hilty, 2002; Restall  
125 *et al.* 2006a, 2006b). The full list of species and number of individuals caught is given in Table  
126 1. In the temperate forest habitat, we sampled birds in a young deciduous forest located in  
127 Sologne (thereafter called Temperate Forest, France, 47°26'N, 2°13'E) during spring 2019 (10  
128 sampling days). At that time of the season, sunrise occurs at 06:00 am and sunset around 09:00  
129 pm. In the tropical forest, we sampled birds in the Amazonian primary rain forest during July  
130 and August 2018 and October 2019 (15 and 11 sampling days in total) in the two field sites of  
131 the Nouragues French research station: Saut-Pararé (thereafter Tropical Forest 1, French  
132 Guyana, 4°02'N, 52°41'W, 8 sampling days in 2018 and 11 sampling days in 2019) and  
133 Inselberg (thereafter Tropical Forest 2, French Guyana, 4°05'N, 52°41'W, 7 sampling days in  
134 2018) where sunrise occurs at 06:00 am and sunset at 06:00 pm. In the first site, we sampled  
135 birds both within the forest undergrowth and at its edge in the open area close to the field camp  
136 (Fig. 1). On one typical sunny day, we measured abiotic parameters at ground level in these  
137 two areas (open and undergrowth): temperature (°C), UV index, luminosity intensity (in lux).  
138 These measures were performed 5 times over the course of the day (08:00 am, 10:00 am, 12:00  
139 am, 15:00 pm, 17:30 pm), using a CROSCAAL phone – Trekker – X4 equipped with a software  
140 X4 sensors in 10 different spots randomly chosen to take spatial heterogeneity into account.

141           For birds that can be kept in captivity throughout their lives such as poultry, the preferred  
142 method to track temporal patterns of oocysts release as accurately as possible is to collect faeces  
143 every 2 h from sunrise to sunset for each bird maintained in an individual cage (Filipiak *et al.*,  
144 2009). However, in field conditions, enclosing wild birds in a cage for an entire day is not  
145 acceptable from an ethical point of view, due to the stress of capture and prolonged restraint,  
146 which may alter both their subsequent survival and ability to reproduce, as birds might have  
147 been breeding at the time of capture. To limit the stress imposed on birds, we followed the  
148 protocol adopted in snow bunting (Dolnik *et al.*, 2011). Birds were placed in individual cages,  
149 the bottom of which was lined with aluminium foil, at any time of capture during the day, for a  
150 maximum of one hour before being released at their site of capture, had the birds shed faeces  
151 or not. All fresh faeces present on the aluminium foil were collected and homogenised with a  
152 small spoon and stored at 4°C in 15 mL Falcon tubes labelled with the date, time of the day and  
153 bird species. To avoid repeated sampling of the same individuals, birds having shed faeces were  
154 marked with a drop of nontoxic nail varnish on the tarsus and in case of recapture, were not  
155 subjected again to faeces collection and were thus immediately released.

156

#### 157 *Prevalence and intensity of parasite infection by Isospora spp.*

158 At the end of each field session in the two habitats (temperate/tropical), faeces samples were  
159 weighted to the nearest 0.01g using an electronic balance and a solution of Sheater (45% sugar  
160 in water) was added to a concentration of 1 mg of faeces for 4 ml of sheater, after which samples  
161 were gently homogenised. We sampled 600 µl of this solution on a McMaster chamber and  
162 allowed oocysts to float to the top for 10 min. All the oocysts of *Isospora* spp. under the grid  
163 of each chamber in the McMaster were counted using 10 x magnification. Two counts were  
164 obtained for each individual faecal sample, and the average concentration, expressed as the



165 number of oocysts per gram of faecal sample, was used as an estimate of oocyst production  
166 rate.

167

### 168 *Statistical analyses*

169 The time of faeces collection was first calculated for each bird as the average hour between  
170 entrance and release from the cage. This time was standardized in the proportion of daily  
171 sunshine duration to take differences in day length between tropical and temperate sites into  
172 account as follow :  $h_{standardized} = \frac{h_{average} - h_{sunrise}}{h_{sunset} - h_{sunrise}}$  with  $h_{average}$  the average hour of  
173 collection,  $h_{sunrise}$  and  $h_{sunset}$  the sunrise and sunset time. Additionally, to compare morning and  
174 afternoon prevalence and infection intensity, daylength was divided into two parts: morning:  
175 standardized hour < 0.5 and afternoon: standardized hour > 0.5. All statistical analyses were  
176 performed with R software (v.4.0.4, R Core Team 2021) implemented with packages *MASS*,  
177 *car* for model comparison for unbalanced design and *emmeans* for post hoc pairwise  
178 comparison tests.

179 Differences in prevalence and parasite load between temperate (Temperate Forest) and  
180 tropical environments (Tropical Forest 1 + Tropical Forest 2) in birds sampled in the forest  
181 (birds caught in the open area in Tropical Forest 1 were excluded from this analysis) were  
182 analyzed using a Generalized Linear Mixed-Effects Model with Binomial distribution (B-  
183 GLMM, using *glmer* function) and Negative Binomial distribution (NB-GLMM, using  
184 *glmmTMB* function) respectively, including either the standardized hour or part of the day,  
185 habitat (temperate vs. tropical) and their interaction as fixed effects. Species identity was  
186 included as a random effect. The slope of fitted lines/curves were provided assorted with their  
187 95% confidence interval from the models. Similar models were run to compare the two sites in  
188 the tropical habitat (i.e. Tropical Forest 1 vs Tropical Forest 2).

189 The same procedures were used to investigate differences in prevalence and parasite  
190 load in birds from open area *versus* surrounding undergrowth in the Tropical Forest 1.

191 Differences in abiotic factors during the day (temperature, UV radiations and  
192 luminosity) between these two habitats were analyzed including the standardized hour of the  
193 day as quadratic effect to test for a potential maximal effect at midday and the location and their  
194 interaction. The temperature was analyzed using a Gaussian GLM (glm function) while UV  
195 radiation and luminosity were analyzed using NB-GLM (glm.nb function).

196 For all GLMs and GLMMs, model comparisons were made using a deviance analysis  
197 for unbalanced design based on the likelihood ratio  $\chi^2$ -statistics or F-ratio for Gaussian GLM to  
198 test the contribution of the variable. Interactions were discarded when non-significant.

199

## 200 **Results**

### 201 *Differences in temporal patterns of parasite prevalence in species from Temperate and Tropical* 202 *Forests*

203 The prevalence of *Isospora* spp. oocysts in bird faeces increased dramatically as the day  
204 progressed for species inhabiting temperate forest to reach a maximum of 90% of infected  
205 **individuals** (slope<sub>Temperate-Forest</sub> [95%CI] = 6.89 [5.27; 8.50]) whereas in the same time the  
206 prevalence for species living in tropical forest remained unchanged around 25% of infected  
207 individuals (slope<sub>Tropical-Forest</sub> [95%CI] = 0.86 [-0.40; 2.12]) (B-GLMM: effect of time:  $\chi^2_{1=}$   
208 69.76,  $P < 0.0001$ , effect of location:  $\chi^2_{1=}$  6.65,  $P < 0.01$ , effect of their interaction:  $\chi^2_{1=}$  33.72,  
209  $P < 0.0001$ )(Fig. 2). The prevalence of *Isospora* spp. oocysts in bird faeces did not differ  
210 between the two tropical forest sites (B-GLMM: effect of time:  $\chi^2_{1=}$  2.47,  $P = 0.12$ , effect of  
211 sites:  $\chi^2_{1=}$  1.70,  $P = 0.19$ , effect of their interaction:  $\chi^2_{1=}$  0.98,  $P = 0.32$ , slope<sub>Tropical-</sub>  
212 Forest1[95%CI] = 0.43 [-1.18; 2.05] and slope<sub>Tropical-Forest2</sub> [95%CI] = 1.87 [-0.42; 4.20]).

213 Complementary analyses of parasite prevalence contrasting morning and afternoon data are  
214 presented in Table 1 and yielded the same results.

215

### 216 *Differences in temporal patterns of infection intensity between species of Temperate and* 217 *Tropical Forest*

218 Pre-analyses showed differences in infection intensity, measured by parasite load in the host  
219 faeces, between morning and afternoon in Temperate Forest (Wilcoxon test:  $W = 983.5$ ,  $P =$   
220  $0.004$ ), and in Tropical Forest 1 (Wilcoxon test:  $W = 206.5$ ,  $P = 0.01$ ) but not in Tropical Forest  
221 2 (Wilcoxon test:  $W = 29.5$ ,  $P = 0.38$ )(mean  $\pm$  s.e. of number of *Isospora* spp. shed in faeces in  
222 the morning *versus* in the afternoon respectively: Temperate Forest:  $35.36 \pm 15.95$  *versus*  
223  $222.81 \pm 46.01$ , Tropical Forest 1:  $29.71 \pm 24.88$  *versus*  $91.06 \pm 44.13$  and Tropical Forest 2:  
224  $27.20 \pm 23.25$  *versus*  $62.33 \pm 37.92$ ). Therefore, analyses of differences in parasite load were  
225 restricted to data from infected birds sampled in the afternoon (excluding birds from the open  
226 area in Tropical Forest 1). Parasite load remained constant all the afternoon in species living in  
227 temperate habitat (slope<sub>Temperate-Forest</sub> [95%CI] =  $-0.002$  [ $-3.01$ ;  $3.01$ ]; mean  $\pm$  s.e. parasite load:  
228  $222.81 \pm 46.01$  oocysts per bird). In contrast, parasite load increased over time from 0 to around  
229 200 oocyst per bird at sunset (slope<sub>Tropical-Forest</sub> [95%CI] =  $5.98$  [ $2.27$ ;  $9.69$ ], mean  $\pm$  s.e. parasite  
230 load:  $80.72 \pm 30.97$  oocysts per bird), (NB-GLMM: effect of time:  $\chi^2_{1} = 0.00$ ,  $P = 1.00$ , effect  
231 of location:  $\chi^2_{1} = 7.86$ ,  $P < 0.01$ , effect of their interaction:  $\chi^2_{1} = 5.84$ ,  $P < 0.05$ ) (Fig. 3). In the  
232 Tropical Forest, the number of oocyst released in bird faeces increased through the afternoon  
233 and did not differ between the two sites and (NB-GLMM: effect of time:  $\chi^2_{1} = 6.94$ ,  $P < 0.01$ ,  
234 effect of sites:  $\chi^2_{1} = 0.14$ ,  $P = 0.71$ , effect of their interaction:  $\chi^2_{1} = 0.02$ ,  $P = 0.89$ , slope<sub>Tropical-</sub>  
235 Forest 1 [95%CI] =  $6.18$  [ $1.73$ ;  $10.6$ ] and slope<sub>Tropical-Forest 2</sub> [95%CI] =  $6.64$  [ $1.36$ ;  $11.9$ ]).

236

237 *Differences in temporal patterns of prevalence and intensity between open field and*  
238 *undergrowth in the Tropical Forest I*

239 Prevalence of *Isospora* infection increased in the open area but remain stable inside the Tropical  
240 Forest throughout the day (B-GLMM: effect of time:  $\chi^2_1 = 4.90$ ,  $P < 0.05$ ; effect of area:  $\chi^2_1 =$   
241  $2.66$ ,  $P = 0.10$ , effect of their interaction:  $\chi^2_1 = 4.30$ ,  $P < 0.05$ ; slope<sub>openarea</sub> [95% CI] =  $8.87$  [ $1.02$ ;  
242  $16.72$ ] versus slope<sub>undergrowth</sub> [95% CI] =  $0.47$  [ $-1.09$ ;  $2.02$ ])(Fig. 4). For example, in the most  
243 abundant species captured in this open area (*Silver-beaked Tanager*), all individuals caught in  
244 the afternoon shed oocyst in their faeces whereas no individual caught in the morning did (Table  
245 1). Considering only infected birds in the afternoon (standardized hour  $> 0.5$ ), parasite load was  
246 higher in the open area than in the undergrowth (NB-GLMM: effect of time:  $\chi^2_1 = 1.08$ ,  $P =$   
247  $0.30$ ; effect of area:  $\chi^2_1 = 5.35$ ,  $P < 0.05$ , mean  $\pm$  s.e. parasite load open area:  $1028.70 \pm 441.20$   
248 oocysts/bird and forest undergrowth:  $91.06 \pm 44.13$  oocysts/bird) (Fig. 5).

249

250 *Comparison of abiotic factors between the forest undergrowth and open area*

251 Variation in temperature showed a curvilinear relationship with time elapsed since sunrise, with  
252 a peak around  $35^\circ\text{C}$  in open area and only around  $30^\circ\text{C}$  in the forest undergrowth between 12:00  
253 and 15:00 (Gaussian GLM: time x area:  $F_{1,94} = 4.88$ ,  $P = 0.03$ , time<sup>2</sup> x area:  $F_{1,94} = 5.20$ ,  $P =$   
254  $0.02$ , Fig. 6a). In open area, UV radiation and luminosity showed a similar relationship with  
255 time from the morning to midday to reach a high intensity of UV radiation and luminosity  
256 intensity before a strong decrease until the sunset. In contrast, in the forest undergrowth UV  
257 radiation and luminosity intensity remains at very low level throughout the day (NB-GLM for  
258 UV radiation: time:  $\chi^2_1 = 56.39$ ,  $P < 0.0001$ ; time<sup>2</sup>:  $\chi^2_1 = 59.99$ ,  $P < 0.0001$ ; location:  $\chi^2_1 =$   
259  $169.08$ ,  $P < 0.0001$ , Fig. 6b and NB-GLM for luminosity: time:  $\chi^2_1 = 52.01$ ,  $P < 0.0001$ ; time<sup>2</sup>:  
260  $\chi^2_1 = 57.30$ ,  $P < 0.0001$ ; location:  $\chi^2_1 = 188.73$ ,  $P < 0.0001$ , Fig. 6c).

261

## 262 Discussion

263 In this study, we expected circadian variation in coccidial oocysts shedding to be found in  
264 temperate, but not in tropical forest habitat, consistent with the hypothesis that this trait in  
265 parasites is maintained by natural selection to avoid desiccation and UV radiation, to which  
266 oocysts are sensitive before sporulation. We predicted that in an environment where solar  
267 radiations reaching the ground are dramatically reduced, like in the dense undergrowth of a  
268 tropical forest, the circadian output of oocysts typically found in bird coccidia would disappear.  
269 Our results clearly confirmed this prediction since circadian variation in coccidian oocysts was  
270 high in the temperate forest (i.e., 90 % of bird were infected in the late afternoon and almost  
271 none in the morning) and absent in the tropical forest (approximately 20% of bird's sheds  
272 oocyst in their faces whatever the hour of trapping). Furthermore, at a smaller spatial scale in  
273 the Tropical Forest 1 site, a similar result was found, as birds captured in the open area showed  
274 a strong circadian variation contrary to bird species from the surrounding undergrowth of the  
275 rain forest.

276 The main goal of this study was to go one step further regarding the adaptive  
277 significance of circadian variation in coccidial oocysts shedding in birds, demonstrated in all  
278 tested species including birds living in the arctic area (Knight *et al.*, 2018 for a review). Ten  
279 years earlier, Martinaud *et al.* (2009) experimentally demonstrated that the release of oocysts  
280 in the late afternoon was an adaptative trait to avoid desiccation and UV radiation, thus reducing  
281 mortality of oocysts in the external environment. Before our study, such a circadian pattern  
282 appeared to be a ubiquitous phenomenon in intestinal bird parasites, since all studies showed  
283 that oocyst shedding peaks in the late afternoon (reviewed in Knight *et al.* 2018). To our  
284 knowledge, this study is the first to document the absence of circadian variation in coccidial  
285 oocysts shedding in a large set of species inhabiting the undergrowth of a rain forest. This  
286 environment is characterized by low luminosity and low UV radiation levels reaching the

287 ground, combined with high soil and air moisture (Chazdon and Fetcher, 1984; Vazquez-Yanes  
288 *et al.*, 1990). All these abiotic characteristics are favourable for the survival of oocysts in host  
289 faeces (Allen and Fetterer, 2002). If temporal variation in the shedding of coccidial oocysts is  
290 a trait that has evolved under a natural selection to avoid desiccation and UV radiation as  
291 commonly thought (Martinaud *et al.*, 2009), such a pattern should not be observed in an  
292 environment where these abiotic selective pressures are relaxed. Contrary to the continuous  
293 multi-layered vegetation canopy of rain forests, the canopy in temperate forests is moderately  
294 dense and allows greater and seasonally varying light penetration (Anderson, 1964; Koizumi  
295 and Oshima, 1985; Szwagrzyk *et al.*, 2001). Moreover, most temperate forests are managed and  
296 affected by human activities, and light intensities reaching the ground may differ greatly  
297 according to the stage in the forest management (Canham and Marks, 1985; Buckley, 1992;  
298 McElrone *et al.*, 2013). For example, in most recent cuts (1 year-old), light intensity was 3.5 to  
299 5 times higher than in uncut plots, and rapidly decreased over time following selection cutting,  
300 especially at ground level (Beaudet *et al.*, 2004). Therefore, temperate forests are exposed to a  
301 stronger and more variable irradiance compared to tropical rain forest. We observed marked  
302 temporal variation in the shedding of coccidial oocysts in birds from the temperate forest,  
303 consistent with previous studies (Knight *et al.*, 2018). On the contrary, bird species living in the  
304 tropical forest did not show this diurnal variation. These results suggest that natural selection  
305 (evolutionary costs and benefits) may indeed shape release rhythms in *Isospora* spp. of birds  
306 like in many other parasite species (Reece *et al.*, 2017).

307 In addition, comparing circadian variation in coccidial oocysts shedding within the  
308 tropical habitat between species exclusively living in the open area and those living in the  
309 surrounding undergrowth further support this hypothesis. This open area was created at the  
310 construction of the field camp, more than 30 years ago, and has since selected bird species  
311 specialized in open habitats. Indeed, species captured in the open field were never caught in the

312 undergrowth, like Silver-beaked Tanager (*Ramphocelus carbo carbo*), Golden-sided Euphonia  
313 (*Euphonia cayennensis*), Violaceous Euphonia (*Euphonia violacea*), Tropical Kingbird  
314 (*Tyrannus melancholicus*), Great Kiskadee (*Pitangus sulphuratus*), Buff throated saltator  
315 (*Saltator maximus*) and Ruffous browed peppershrike (*Cyclarhis gujanensis*). A review of the  
316 literature (Handbook of the Birds of the World Series) confirms that these bird species are  
317 specialists of open habitats. Unlike the surrounding undergrowth, this open area is characterized  
318 by strong level of luminosity and UV radiation reaching the ground. Indeed, during a typical  
319 day in the tropical environment, light intensity and UV radiation reaching the ground measured  
320 in the open area were on average 100 times higher compared to the ground in the undergrowth,  
321 at any time but in particular at midday. Furthermore, the field camp is located near the equator,  
322 a place where the annual UV doses reach their maximum (Marks and Plewig, 1991), increasing  
323 the selective pressure towards a marked circadian variation in coccidial oocysts shedding.  
324 Therefore, *Isospora* sp. infecting birds living in this open area have likely been selected to evolve  
325 strong temporal variation in oocysts shedding allowing oocyst survival in such a harsh external  
326 environment.

327         Regarding variation in the intensity of *Isospora* spp. infection, we found that parasite  
328 load remained stable in temperate species through the day while it increased in tropical areas,  
329 to reach by the end of the afternoon approximately the same parasite load as in temperate  
330 species. This increase in oocyst release during the day confirms previous results on two species  
331 (*Serinus serinus* and *Sylvia borin*) in which coccidial parasite load in the faeces peaked in the  
332 late afternoon (Lopez *et al.*, 2007). The mechanisms underlying this process, however, are still  
333 unclear. After different phases of multiplication inside the intestinal tractus of its host, oocysts  
334 are released from its host cell and shed into the environment with the faeces. The proximal  
335 factors triggering coccidian oocyst discharge are not identified yet but it is known that they are  
336 linked to host physiology (Boughton, 1933; Dolnik, 1999a, 1999b) and potentially mediated by

337 melatonin, which regulates daily activity patterns in vertebrates. Indeed, outside polar regions  
338 with constant daylight, avian *Isoospora* species use cyclic changes in melatonin concentration in  
339 their host blood to synchronize oocyst output (Dolnik *et al.* 2011, and references therein).  
340 Therefore, we may hypothesize that the threshold of physiological signals inducing oocyst  
341 discharge is very low for tropical species inhabiting the dense undergrowth, allowing oocysts  
342 discharge early in the morning. The accumulation of these signals as the day progressed may  
343 induce the slight but continuous increase in parasite load observed in our data over the  
344 afternoon. Another but not exclusive hypothesis would be that the amount of faeces produced  
345 could be higher in the late afternoon allowing a higher discharge in oocyst in the same time.

346         Finally, our results meet an interesting methodology issue for researchers interested in  
347 working on intestinal parasites like *Isoospora* spp. in wild birds' species (Biard *et al.*, 2015,  
348 2010). Due to circadian rhythm in oocyst shedding in temperate habitat there is a large  
349 difference in the number of *Isoospora* sp. oocysts shed between the morning and afternoon for a  
350 given individual (Filipiak *et al.*, 2009). Hence, to accurately assess, and make meaningful  
351 comparisons in, the prevalence of *Isoospora* infection, sampling of faeces must be standardized  
352 at the same time of the day, as already demonstrated in previous studies (Filipiak *et al.*, 2009;  
353 Dolnik *et al.*, 2010). In the tropical rain forest where this circadian rhythm in oocyst shedding  
354 is absent, prevalence of infection may be assessed from faeces collected at any time of the day.  
355 This makes it easier to work on the effects of intestinal parasites on bird host life-history traits  
356 such as sexually selected traits for instance (Brawner *et al.*, 2000; Hōrak *et al.*, 2004). However,  
357 as parasite load increases as the day progresses, we emphasise the need to precisely record the  
358 time of day at which samples are collected. If *Isoospora* spp. parasite loads are to be compared,  
359 then sampling time should be standardized at the same time of day.

360

361



362 **Concluding remarks**

363 We believe that studying tropical birds improved our knowledge of the ecology of *Isospora*  
364 spp. in natural populations of hosts and the adaptive significance of daily rhythm in oocyst  
365 shedding. The available information on intestinal parasites in tropical birds to date is scarce as  
366 research has often focused on blood parasites (Ricklefs, 1992; Durrant *et al.*, 2006; Ricklefs  
367 and Sheldon, 2007; Merino *et al.*, 2008; Svensson-Coelho *et al.*, 2014). Our study clearly  
368 showed that the knowledge on the interaction between parasite and host in the temperate area  
369 cannot be transposed for birds in tropical area. Therefore, future investigations are needed to  
370 reveal more information's on host-parasite interactions concerning the probability to be infected  
371 in the two different ecosystems. Comparative analysis with a large set of species would be very  
372 useful to investigate the ecological factors and host life-history traits underlying parasite  
373 diversity across different ecological contexts (tropical *versus* temperate).

374

375 **Supplementary material**

376 No supplementary material

377

378 **Data**

379 Once the paper has been accepted, data will be deposited in the Dryad

380

381 **Acknowledgements**

382 This work has been conducted within the French natural reserve of Nouragues. We thank the  
383 Nouragues CNRS research field station (USR mixte LEEISA, CNRS; Cayenne) and the staff  
384 dedicated. We would like to thank all the people who contributed to the fieldwork.

385

386

387 **Author Contribution**

388 CB, KM and JM conceived and designed the study. CB, MT, SM, SBA, LD and JM conducted  
389 data gathering. KM and JM performed statistical analyses. CB, KM and JM wrote the article.

390

391 **Financial Support**

392 We thank the Nouragues CNRS research field station, which benefits from “Investissement  
393 d'Avenir” grants managed by Agence Nationale de la Recherche (AnaEE France ANR-11-  
394 INBS-0001; Labex CEBA ANR-10-LABX-25-01).

395

396 **Conflicts of Interest**

397 The authors declare there are no conflicts of interest.

398

399 **Ethical Standards**

400 This work conforms to French legal requirements, and accepted international ethical standards,  
401 including those relating to conservation and welfare, and to the journal’s policy on these  
402 matters. We thank the Direction Régionale de l’Environnement, de l’Aménagement et du  
403 Logement (DREAL) de la Guyane, de la Nouvelle-Aquitaine et du Loir et Cher for delivering  
404 legal authorizations of catching birds (authorizations no: R03-2018-07-17-002, R03-2019-03-  
405 14-005, 128/2017, 2019D/2323, 41-2018-04-03-002).

406

407

408

409

410

411

412 **References**

413 **Abd-Al-Aal, Z, Ramadan, NF and Al-Hoot, A** (2000) Life-cycle of *Isospora mehlhornii* sp.  
414 nov. (Apicomplexa: Eimeriidae), parasite of the Egyptian swallow *Hirundo rubicola*  
415 *savignii*. *Parasitology Research* **86**, 270-278.

416

417 **Allen, PC and Fetterer, RH** (2002) Recent advances in biology and immunobiology of  
418 *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of  
419 poultry. *Clinical and Microbiology Review* **15**, 58-65.

420

421 **Anderson, MC** (1964) Studies of the woodland light climate: II. Seasonal variation in the light  
422 climate. *Journal of Ecology* **52**, 643-663.

423

424 **Beaudet, M, Messier, C and Leduc, A** (2004) Understorey light profiles in temperate  
425 deciduous forests: recovery process following selection cutting. *Journal of Ecology* **92**,  
426 328-338.

427

428 **Benjamini, Y and Hochberg, Y** (1995) Controlling the false discovery rate: a practical and  
429 powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B* **57**,  
430 289-300.

431

432 **Biard, C, Monceau, K, Motreuil, S and Moreau, J** (2015) Interpreting immunological  
433 indices: The importance of taking parasite community into account. An example in  
434 blackbirds *Turdus merula*. *Methods in Ecology and Evolution* **6**, 960-972.

435

436 **Biard, C, Saulnier, N, Gaillard, M and Moreau, J** (2010) Carotenoid-based bill colour is an  
437 integrative signal of multiple parasite infection in blackbird. *Naturwissenschaften* **97**, 987-  
438 995.

439

440 **Boughton, DC** (1933) Diurnal gametic periodicity in avian *Isospora*. *American Journal of*  
441 *Hygien* **18**, 161-184.

442

443 **Bourgeron, PS** (1983) Spatial aspects of vegetation structure. In Golley FB (eds). *Tropical*  
444 *rain forest ecosystems: structure and function. Ecosystems of the world*. Amsterdam and  
445 New York, Elsevier Scientific Pub. Co., pp. 29-47.

446

447 **Brawner, WR and Hill, GE** (1999) Temporal variation in shedding of coccidial oocysts:  
448 implications for sexual-selection studies. *Canadian Journal of Zoology* **77**, 347-350.

449

450 **Brawner, WR, Hill, GE and Sundermann, CA** (2000) Effects of coccidial and mycoplasma  
451 infections on carotenoid-based plumage pigmentation in male House finches. *The Auk* **117**,  
452 952-963.

453

454 **Brinkmann, WLF** (1971) Light environment in a tropical rain forest of central Amazonia. *Acta*  
455 *Amazonica* **1**, 37-49.

456

457 **Brown, M, Ball, S and Holman, D** (2001) The periodicity of isosporan oocyst discharge in the  
458 greenfinch (*Carduelis chloris*). *Journal of Natural History* **35**, 945-948.

459

460 **Bruno, RD, Rocha, HR, Freitas, HC, Goulden, ML and Miller, SD** (2006) Soil moisture  
461 dynamics in an eastern Amazonian tropical forest. *Hydrology Process* **20**, 2477-2489.  
462

463 **Buckley, GP** (1992) *Ecology and management of coppice woodlands*. London, UK: Chapman  
464 and Hall.  
465

466 **Bush, AO, Fernández, JC, Esch, GW and Seed, JR** (2001) *Parasitism: the diversity and*  
467 *ecology of animal parasites*. Cambridge, UK: Cambridge University Press.  
468

469 **Canham, CD and Marks, PL** (1985) The response of woody plants to disturbance: patterns of  
470 establishment and growth. In Pickett STA and White PS (eds). *The Ecology of Natural*  
471 *Disturbance and Patch Dynamics*. New York, USA: Academic Press, pp. 197-216.  
472

473 **Chazdon, RL and Fetcher, N** (1984) Photosynthetic light environments in a lowland tropical  
474 rain forest in Costa Rica. *Journal of Ecology* **72**, 553-564.  
475

476 **Combes, C** (2001) *Parasitism: The ecology and evolution of intimate interactions*. London,  
477 UK: University of Chicago Press and London.  
478

479 **Combes, C, Fournier, A, Moné, H and Théron, A** (1994) Behaviours in trematode cercariae  
480 that enhance parasite transmission: patterns and processes. *Parasitology* **109**, S3-S13.  
481

482 **Dolnik, OV** (1999a) Diurnal periodicity of oocysts release of *Isospora dilatata* (Sporozoa:  
483 Eimeriidae) from the common Starling (*Sturnus vulgaris*) in nature. *Parasitologia* **33**, 74-  
484 80.

485

486 **Dolnik, OV** (1999b) Diurnal periodicity in appearance of *Isospora* (Protozoa: Coccidea)  
487 oocysts from some passerine birds. *Proceedings of the Zoological Institute RAS* **281**, 113-  
488 118.

489

490 **Dolnik, OV, Dolnik, VR and Bairlein, F** (2010) The effect of host foraging ecology on the  
491 prevalence and intensity of coccidian infection in wild passerine birds. *Ardea* **98**, 97-103.

492

493 **Dolnik, OV, Metzger, BJ and Loonen, MJJE** (2011) Keeping the clock set under the  
494 midnight sun: diurnal periodicity and synchrony of avian *Isospora* parasites cycle in the  
495 High Arctic. *Parasitology* **138**, 1077-1081.

496

497 **Durrant, KL, Beadell, JS, Ishtiaq, F, Graves, GR, Olson, SL, Gering, E, Peirce, MA,**  
498 **Milensky, CM, Schmidt, BK, Gebhard, C and Fleischer, RC** (2006) Avian hematozoa  
499 in South America: A comparison of temperate and tropical zones. *Ornithological*  
500 *Monographs* **60**, 98-111.

501

502 **Filipiak, L, Mathieu, F and Moreau, J** (2009) Caution on the assessment of intestinal parasitic  
503 load in studying parasite-mediated sexual selection: The case of blackbirds coccidiosis.  
504 *International Journal for Parasitology* **39**, 741-746.

505

506 **Freiberg, M** (1997) Spatial and temporal pattern of temperature and humidity of a tropical  
507 premontane rain forest tree in Costa Rica. *Selbyana* **18**, 77-84.

508

509 **Hilty, S** (2002) *Birds of Venezuela*, 2<sup>nd</sup> Edn. Princeton, USA: Princeton University Press

510

511 **Hörak, P, Saks, L, Karu, U, Ots, I, Surai, PF and McGraw, KJ** (2004) How coccidian  
512 parasites affect health and appearance of greenfinches. *Journal of Animal Ecology* **73**, 935-  
513 947.

514

515 **Hudman, SP, Ketterson, ED and Nolan, V** (2000) Effects of time of sampling on oocyst  
516 detection and effects of age and experimentally elevated testosterone on prevalence of  
517 coccidia in male dark-eyed juncos. *The Auk* **117**, 1048-1051.

518

519 **Knight, A, Ewen, JG, Brekke, P and Santure, AW** (2018) Chapter Two - The Evolutionary  
520 Biology, Ecology and Epidemiology of Coccidia of Passerine Birds. In Rollinson D and  
521 Stothard JR (eds). *Advances in Parasitology*. Academic Press, pp. 35-60.

522

523 **Koizumi, H and Oshima, Y** (1985) Seasonal changes in photosynthesis of four understory  
524 herbs in deciduous forests. *Botanical Magazine* **98**, 1-13.

525

526 **Kruszewicz, AG** (1995) The occurrence of *Isoospora lacazei* (Coccidia: Eimeriidae) and its  
527 influence on nestling growth in House Sparrows (*Passer domesticus*) and Tree Sparrows  
528 (*Passer montanus*). In Pinowski J, Kavanagh BP and Pinowska B (eds). *Nestling mortality*  
529 *of granivorous birds due to microorganisms and toxic substances: Synthesis*. Warszawa,  
530 Poland: Polish Scientific Publishers, pp. 291-305.

531

532 **Lopez, G, Figuerola, J and Soriguer, R** (2007) Time of day, age and feeding habits influence  
533 coccidian oocyst shedding in wild passerines. *International Journal for Parasitology* **37**,  
534 559-564.

535

536 **Marks, R and Plewig, G** (1991) The environmental threat to the skin. London, UK: Taylor  
537 and Francis Ltd.

538

539 **Martinaud G., Billaudelle M and Moreau, J** (2009) Circadian variation in shedding of the  
540 oocysts of *Isospora turdi* (Apicomplexa) in blackbirds (*Turdus merula*): An adaptative trait  
541 against desiccation and ultraviolet radiation. *International Journal for Parasitology* **39**,  
542 735-739.

543

544 **McElrone AJ, Choat B, Gambetta GA and Brodersen CR** (2013) Water uptake and transport  
545 in vascular plants. *Nature Education Knowledge* **4(5)**:6.

546

547 **Merino, S, Moreno, J, Vásquez, RA, Martínez, J, Sánchez-Monsálvez, I, Estades, CF, Ippi,**  
548 **S, Sabat, P, Rozzi, R and McGehee, S** (2008) Haematozoa in forest birds from southern  
549 Chile: Latitudinal gradients in prevalence and parasite lineage richness. *Austral Ecology*  
550 **33**, 329-340.

551

552 **Misof, K** (2004) Diurnal cycle of *Isospora* spp. oocyst shedding in Eurasian blackbirds (*Turdus*  
553 *merula*). *Canadian Journal of Zoology* **82**, 764-768.

554

555 **Olsen, OW** (1974) Animal parasites: their life cycles and ecology. Baltimore, USA: University  
556 Park Press.

557

558 **R Core Team** (2021). R: A language and environment for statistical computing. R Foundation  
559 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>



560

561 **Reece, SE, Prior, KF and Mideo, N** (2017) The life and times of parasites: Rhythms in  
562 strategies for within-host survival and between-host transmission. *Journal of Biological*  
563 *Rhythms* **32**, 516-533.

564

565 **Restall, R, Rodner, C and Lentino, M** (2006a) *Birds of Northern South America: An*  
566 *Identification Guide. Species Accounts*, vol. 1, 1st Edn. Helm Field Guides.

567

568 **Restall, R, Rodner, C and Lentino, M** (2006b) *Birds of Northern South America: An*  
569 *Identification Guide. Plates and maps*, vol. 2, 1st Edn. Helm Field Guides.

570

571 **Ricklefs, RE** (1992) Embryonic development period and the prevalence of avian blood  
572 parasites. *Proceedings of the National Academy of Sciences* **89**, 4722-4725.

573

574 **Ricklefs, RE and Sheldon, KS** (2007) Malaria prevalence and white-blood-cell response to  
575 infection in a tropical and in a temperate thrush. *The Auk* **124**, 1254-1266.

576

577 **Svensson-Coelho, M, Ellis, VA, Loiselle, BA, Blake, JG and Ricklefs, RE** (2014) Reciprocal  
578 specialization in multihost malaria parasite communities of birds: a temperate-tropical  
579 comparison. *American Naturalist* **184**, 624-635.

580

581 **Szwagrzyk, J, Szewczyk, J and Bodziarczyk, J** (2001) Dynamics of seedling banks in beech  
582 forest: results of a 10-year study on germination, growth and survival. *Forest Ecology and*  
583 *Management* **141**, 237-250.

584

585 **Théron, A** (1984) Early and late shedding patterns of *Schistosoma mansoni* Cercariae:  
586 Ecological significance in transmission to human and murine hosts. *Journal of*  
587 *Parasitology* **70**, 652-655.

588

589 **Vazquez-Yanes, C, Orozco-Segovia, A, Rincon, E, Sanchez-Coronado, ME, Huante, P,**  
590 **Toledo, JR and Barradas, VL** (1990) Light beneath the litter in a tropical forest: Effect  
591 on seed germination. *Ecology* **71**, 1952-1958.

592

593

594

595

596

597

598

599

600

601

602

603

604

605

606

607

608

609

610 Table 1: Complete list of bird species included in this study, showing prevalence of infection  
611 by *Isospora* spp. (total number of birds caught and number of birds infected in brackets)  
612 separately for birds caught in the morning (h standardized < 0.5) and in the afternoon (h  
613 standardized > 0.5). Birds were caught in three different environments: Temperate Forest  
614 (Souesmes), Tropical Forest 1 (Nouragues, Pararé) undergrowth and open area, Tropical Forest  
615 2 (Nouragues, Inselberg). Differences in parasite prevalence in host faeces between morning  
616 and afternoon differed between tropical and temperate species (B-GLMM: effect of morning  
617 vs. afternoon:  $\chi^2_1 = 76.21$ ,  $P < 0.0001$ , effect of the site:  $\chi^2_1 = 55.41$ ,  $P < 0.0001$ , effect of their  
618 interaction:  $\chi^2_1 = 38.03$ ,  $P < 0.0001$ ). In the Temperate Forest, the prevalence of *Isospora* was  
619 higher in the afternoon than in the morning, whereas prevalence did not differ between morning  
620 and afternoon in the Tropical Forest (Table 2).

| Species                            | Standardized hour          |                 |         |
|------------------------------------|----------------------------|-----------------|---------|
|                                    | [0:0,5[                    | [0,5:1]         |         |
| <b>TEMPERATE FOREST - Souesmes</b> | <b>99 (14)</b>             | <b>112 (95)</b> |         |
| <b>Acrocephalidae</b>              |                            |                 |         |
| <i>Hippolais polyglotta</i>        | Melodious warbler          | 1 (1)           | 4 (4)   |
| <b>Aegithalidae</b>                |                            |                 |         |
| <i>Aegithalos caudatus</i>         | Long-tailed tit            | 1 (0)           | 4 (4)   |
| <b>Certhiidae</b>                  |                            |                 |         |
| <i>Certhia brachydactyla</i>       | Short-toed tree creeper    | 4 (0)           | 2 (1)   |
| <b>Emberizidae</b>                 |                            |                 |         |
| <i>Emberiza citrinella</i>         | Yellowhammer               | 0               | 1 (1)   |
| <b>Fringillidae</b>                | <b>11 (1)</b>              | <b>12 (10)</b>  |         |
| <i>Fringilla coelebs</i>           | Common chaffinch           | 5 (1)           | 7 (7)   |
| <i>Carduelis cannabina</i>         | Common linnet              | 1 (0)           | 0       |
| <i>Pyrhula pyrrhula</i>            | Eurasian bullfinch         | 2 (0)           | 5 (3)   |
| <i>Carduelis chloris</i>           | European greenfinch        | 3 (0)           | 0       |
| <b>Locustellidae</b>               |                            |                 |         |
| <i>Locustella naevia</i>           | Common grasshopper-warbler | 2 (0)           | 0       |
| <b>Motacillidae</b>                |                            |                 |         |
| <i>Anthus trivialis</i>            | Tree pipit                 | 0               | 2 (2)   |
| <b>Muscicapidae</b>                | <b>15 (1)</b>              | <b>8 (8)</b>    |         |
| <i>Phoenicurus ochruros</i>        | Black redstart             | 1 (0)           | 0       |
| <i>Luscinia megarhynchos</i>       | Common nightingale         | 2 (0)           | 2 (2)   |
| <i>Phoenicurus phoenicurus</i>     | Common redstart            | 0               | 1 (1)   |
| <i>Erithacus rubecula</i>          | European robin             | 11 (1)          | 4 (4)   |
| <i>Saxicola torquatus</i>          | European stonechat         | 1 (0)           | 1 (1)   |
| <b>Paridae</b>                     | <b>17 (5)</b>              | <b>25 (17)</b>  |         |
| <i>Parus caeruleus</i>             | Eurasian blue tit          | 9 (1)           | 14 (11) |
| <i>Parus major</i>                 | Great tit                  | 6 (3)           | 8 (4)   |
| <i>Parus palustris</i>             | Marsh tit                  | 2 (1)           | 3 (2)   |
| <b>Passeridae</b>                  |                            |                 |         |
| <i>Passer domesticus</i>           | House sparrow              | 3 (0)           | 11 (11) |
| <b>Phylloscopidae</b>              | <b>10 (0)</b>              | <b>9 (6)</b>    |         |
| <i>Phylloscopus collybita</i>      | Common chiffchaff          | 10 (0)          | 8 (6)   |
| <i>Phylloscopus trochilus</i>      | Willow warbler             | 0               | 1 (0)   |
| <b>Picidae</b>                     |                            |                 |         |
| <i>Jynx torquilla</i>              | Eurasian wryneck           | 1 (0)           | 2 (2)   |
| <b>Prunellidae</b>                 |                            |                 |         |
| <i>Prunella modularis</i>          | Dunnock                    | 3 (1)           | 3 (1)   |
| <b>Sittidae</b>                    | <b>6 (1)</b>               | <b>1 (1)</b>    |         |
| <i>Sitta europaea</i>              | Eurasian nuthatch          | 4 (1)           | 1 (1)   |

|   |                               |                |                |
|---|-------------------------------|----------------|----------------|
| <i>Sturnus vulgaris</i>                         | Common starling               | 2 (0)          | 0              |
| <b>Sylviidae</b>                                |                               | <b>24 (4)</b>  | <b>26 (25)</b> |
| <i>Sylvia communis</i>                          | Common whitethroat            | 4 (0)          | 9 (9)          |
| <i>Sylvia atricapilla</i>                       | Eurasian blackcap             | 17 (4)         | 13 (12)        |
| <i>Sylvia borin</i>                             | Garden warbler                | 3 (0)          | 4 (4)          |
| <b>Troglodytidae</b>                            |                               |                |                |
| <i>Troglodytes troglodytes</i>                  | Eurasian wren                 | 0              | 1 (1)          |
| <b>Turdidae</b>                                 |                               |                |                |
| <i>Turdus philomelos</i>                        | Song thrush                   | 1 (0)          | 1 (1)          |
| <b>TROPICAL FOREST 1 - Pararé</b>               |                               | <b>79 (17)</b> | <b>74 (17)</b> |
| <b>Cardinalidae</b>                             |                               |                |                |
| <i>Cyanocopsa cyanooides</i>                    | Blue-black grosbeak           | 1 (0)          | 0              |
| <b>Columbidae</b>                               |                               |                |                |
| <i>Leptotila rufaxilla</i>                      | Grey-fronted dove             | 0              | 1 (0)          |
| <b>Cotingidae</b>                               |                               |                |                |
| <i>Lipaugus vociferans</i>                      | Screaming piha                | 1 (0)          | 1 (0)          |
| <b>Formicariidae</b>                            |                               |                |                |
| <i>Formicarius analis</i>                       | Black-faced antthrush         | 1 (0)          | 0              |
| <b>Furnariidae</b>                              |                               | <b>14 (3)</b>  | <b>14 (5)</b>  |
| <i>Xiphorhynchus pardalotus pardalotus</i>      | Chestnut-rumped woodcreeper   | 1 (0)          | 1 (1)          |
| <i>Automolus infuscatus</i>                     | Olive-backed foliage-gleaner  | 4 (1)          | 0              |
| <i>Xenops minutus</i>                           | White-throated xenops         | 0              | 1 (0)          |
| <i>Philydor erythrocerum</i>                    | Rufous-rumped foliage-gleaner | 1 (0)          | 0              |
| <i>Sclerurus rufigularis</i>                    | Short-billed leaftosser       | 2 (1)          | 0              |
| <i>Glyphorhynchus spirurus spirurus</i>         | Wedge-billed woodcreeper      | 6 (1)          | 12 (4)         |
| <b>Grallariidae</b>                             |                               |                |                |
| <i>Hylopezus macularius</i>                     | Spotted antpitta              | 0              | 1 (0)          |
| <b>Pipridae</b>                                 |                               | <b>9 (1)</b>   | <b>14 (0)</b>  |
| <i>Pipra erythrocephala</i>                     | Golden-headed manakin         | 1 (0)          | 0              |
| <i>Manacus manacus manacus</i>                  | White-bearded manakin         | 1 (1)          | 2 (0)          |
| <i>Dixiphia pipra pipra</i>                     | White-crowned manakin         | 2 (0)          | 7 (0)          |
| <i>Lepidothrix serena</i>                       | White-fronted manakin         | 2 (0)          | 3 (0)          |
| <i>Corapipo gutturalis</i>                      | White-throated manakin        | 3 (0)          | 2 (0)          |
| <b>Poliptilidae</b>                             |                               |                |                |
| <i>Ramphocaenus melanurus</i>                   | Long-billed gnatwren          | 1 (0)          | 0              |
| <b>Thamnophilidae</b>                           |                               | <b>29 (8)</b>  | <b>26 (8)</b>  |
| <i>Hypocnemoides melanopogon</i>                | Black-chinned antbird         | 0              | 1 (0)          |
| <i>Percnostola rufifrons rufifrons</i>          | Black-headed antbird          | 4 (0)          | 0              |
| <i>Frederickena viridis</i>                     | Black-throated antshrike      | 1 (0)          | 0              |
| <i>Myrmotherula (epinecrophylla) gutturalis</i> | Brown bellied antwren         | 4 (2)          | 1 (1)          |
| <i>Thamnomanes caesius</i>                      | Cinereous antshrike           | 1 (0)          | 3 (1)          |

|   |                             |              |                |
|---|-----------------------------|--------------|----------------|
| <i>Willisornis poecilinotus</i>             | Common scale-backed antbird | 2 (1)        | 1 (0)          |
| <i>Thamnomanes ardesiacus</i>               | Dusky-throated antshrike    | 0            | 2 (2)          |
| <i>Cercomacra cinerans</i>                  | Grey antbird                | 0            | 1 (0)          |
| <i>Myrmotherula menetriesii</i>             | Grey antwren                | 1 (0)        | 4 (2)          |
| <i>Myrmotherula longipennis</i>             | Long-winged antwren         | 0            | 5 (0)          |
| <i>Thamnophilus murinus</i>                 | Mouse-colored antshrike     | 2 (0)        | 0              |
| <i>Iseria guttata</i>                       | Rufous-bellied antwren      | 2 (1)        | 1 (0)          |
| <i>Hylophylax naevius naevius</i>           | Spot-backed antbird         | 2 (1)        | 0              |
| <i>Hypocnemis cantator</i>                  | Warbling-antbird            | 1 (1)        | 0              |
| <i>Myrmotherula axillaris</i>               | White-flanked antwren       | 2 (0)        | 2 (0)          |
| <i>Pithys albifrons albifrons</i>           | White-plumed antbird        | 7 (2)        | 5 (2)          |
| <b>Thraupidae</b>                           |                             | <b>4 (0)</b> | <b>0</b>       |
| <i>Tachyphonus surinamus surinamus</i>      | Fulvous-crested tanager     | 2 (0)        | 0              |
| <i>Lanio fulvus</i>                         | Fulvous shrike-tanager      | 2 (0)        | 0              |
| <b>Tityridae</b>                            |                             | <b>5 (0)</b> | <b>3 (1)</b>   |
| <i>Onychorhynchus coronatus</i>             | Royal Flycatcher            | 0            | 1 (0)          |
| <i>Terenotriccus erythrus</i>               | Ruddy-tailed flycatcher     | 1 (0)        | 0              |
| <i>Myiobius barbatus</i>                    | Sulphur-rumped flycatcher   | 4 (0)        | 2 (1)          |
| <b>Troglodytidae</b>                        |                             |              |                |
| <i>Pheugopedius coraya</i>                  | Coraya wren                 | 3 (1)        | 0              |
| <b>Turdidae</b>                             |                             |              |                |
| <i>Turdus albicollis</i>                    | White-necked thrush         | 4 (1)        | 0              |
| <b>Tyrannidae</b>                           |                             | <b>6 (2)</b> | <b>13 (3)</b>  |
| <i>Platyrhynchus saturatus</i>              | Cinamon-crested spadebill   | 0            | 1 (0)          |
| <i>Platyrhynchus coronatus</i>              | Golden-crowned spadebill    | 1 (0)        | 1 (0)          |
| <i>Mionectes macconnelli</i>                | McConnell's flycatcher      | 5 (2)        | 6 (2)          |
| <i>Rhynchocyclus olivaceus</i>              | Olivaceous flatbill         | 0            | 3 (1)          |
| <i>Corythopsis torquatus</i>                | Ringed antpipit             | 0            | 2 (0)          |
| <b>Vireonidae</b>                           |                             |              |                |
| <i>Hylophilus ochraceiceps</i>              | Tawny-crowned greenlet      | 1 (1)        | 1 (0)          |
| <b>TROPICAL FOREST 1 OPEN AREA - Pararé</b> |                             | <b>9 (0)</b> | <b>16 (10)</b> |
| <b>Fringillidae</b>                         |                             | <b>0</b>     | <b>4 (3)</b>   |
| <i>Euphonia cayennensis</i>                 | Golden-sided euphonia       | 0            | 1 (1)          |
| <i>Euphonia violacea</i>                    | Violaceous euphonia         | 0            | 3 (2)          |
| <b>Thraupidae</b>                           |                             | <b>6 (0)</b> | <b>9 (7)</b>   |
| <i>Coereba flaveola</i>                     | Bananaquit                  | 0            | 1 (1)          |
| <i>Thraupis episcopus</i>                   | Blue-grey tanager           | 0            | 1 (0)          |
| <i>Saltator maximus</i>                     | Buff-throated saltator      | 1 (0)        | 0              |
| <i>Sporophila lineola</i>                   | Lined seedeater             | 0            | 1 (0)          |
| <i>Ramphocelus carbo carbo</i>              | Silver-beaked tanager       | 5 (0)        | 6 (6)          |
| <b>Tyrannidae</b>                           |                             | <b>2 (0)</b> | <b>2 (0)</b>   |
| <i>Pitangus sulphuratus</i>                 | Great kiskadee              | 1 (0)        | 0              |

|  |                                  |               |               |
|--|----------------------------------|---------------|---------------|
| <i>Tyrannus melancholicus</i>              | Tropical kingbird                | 1 (0)         | 2 (0)         |
| <b>Vireonidae</b>                          |                                  |               |               |
| <i>Cyclarhis gujanensis</i>                | Rufous-browed peppershrike       | 1 (0)         | 1 (0)         |
| <b>TROPICAL FOREST 2- Inselberg</b>        |                                  | <b>49 (5)</b> | <b>40 (9)</b> |
| <b>Caprimulgidae</b>                       |                                  |               |               |
| <i>Caprimulgus nigrescens</i>              | Blackish nightjar                | 0             | 1 (1)         |
| <b>Cardinalidae</b>                        |                                  |               |               |
| <i>Cyanocompsa cyanooides</i>              | Blue-black grosbeak              | 1 (0)         | 0             |
| <b>Furnariidae</b>                         |                                  | <b>8 (3)</b>  | <b>4 (4)</b>  |
| <i>Xiphorhynchus pardalotus pardalotus</i> | Chestnut-rumped woodcreeper      | 1 (0)         | 0             |
| <i>Deconychura longicauda</i>              | Northern long-tailed woodcreeper | 1 (1)         | 1 (1)         |
| <i>Certhiasomus stictolaemus</i>           | Spot-throated woodcreeper        | 0             | 1 (1)         |
| <i>Glyphorhynchus spirurus spirurus</i>    | Wedge-billed woodcreeper         | 6 (2)         | 2 (2)         |
| <b>Galbulidae</b>                          |                                  |               |               |
| <i>Galbula albirostris</i>                 | Yellow-billed jacamar            | 1 (0)         | 0             |
| <b>Pipridae</b>                            |                                  | <b>17 (0)</b> | <b>10 (0)</b> |
| <i>Manacus manacus manacus</i>             | White-bearded manakin            | 1 (0)         | 0             |
| <i>Dixiphia pipra pipra</i>                | White-crowned manakin            | 7 (0)         | 4 (0)         |
| <i>Lepidothrix serena</i>                  | White-fronted manakin            | 7 (0)         | 2 (0)         |
| <i>Corapipo gutturalis</i>                 | White-throated manakin           | 2 (0)         | 4 (0)         |
| <b>Poliophtilidae</b>                      |                                  |               |               |
| <i>Microbates collaris</i>                 | Collared gnatwren                | 1 (0)         | 0             |
| <b>Thamnophilidae</b>                      |                                  | <b>12 (2)</b> | <b>15 (3)</b> |
| <i>Percnastola rufifrons rufifrons</i>     | Black-headed antbird             | 1 (0)         | 0             |
| <i>Willisornis poecilinotus</i>            | Common scale-backed antbird      | 1 (0)         | 4 (0)         |
| <i>Thamnomanes ardesiacus</i>              | Dusky-throated antshrike         | 2 (1)         | 1 (1)         |
| <i>Myrmotherula menetriesii</i>            | Grey antwren                     | 1 (0)         | 1 (0)         |
| <i>Myrmotherula longipennis</i>            | Long-winged antwren              | 1 (0)         | 4 (2)         |
| <i>Thamnophilus murinus</i>                | Mouse-colored antshrike          | 1 (0)         | 0             |
| <i>Hylophylax naevius naevius</i>          | Spot-backed antbird              | 1 (0)         | 0             |
| <i>Myrmotherula axillaris</i>              | White-flanked antwren            | 0             | 1 (0)         |
| <i>Pithys albifrons albifrons</i>          | White-plumed antbird             | 4 (1)         | 4 (0)         |
| <b>Thraupidae</b>                          |                                  | <b>0</b>      | <b>3 (0)</b>  |
| <i>Tachyphonus surinamus surinamus</i>     | Fulvous-crested tanager          | 0             | 2 (0)         |
| <i>Saltator grossus</i>                    | Slate-colored grosbeak           | 0             | 1 (0)         |
| <b>Tityridae</b>                           |                                  | <b>2 (0)</b>  | <b>1 (0)</b>  |
| <i>Schoffornis turdina</i>                 | Brown-winged schiffornis         | 1 (0)         | 0             |
| <i>Myiobius barbatus</i>                   | Sulphur-rumped flycatcher        | 1 (0)         | 1 (0)         |
| <b>Turdidae</b>                            |                                  |               |               |
| <i>Turdus albicollis</i>                   | White-necked thrush              | 4 (0)         | 1 (1)         |
| <b>Tyrannidae</b>                          |                                  |               |               |
| <i>Mionectes macconnelli</i>               | McConnell's flycatcher           | 3 (0)         | 4 (0)         |

**Vireonidae**

|                                |                        |   |       |
|--------------------------------|------------------------|---|-------|
| <i>Hylophilus ochraceiceps</i> | Tawny-crowned greenlet | 0 | 1 (0) |
| 621                            |                        |   |       |
| 622                            |                        |   |       |
| 623                            |                        |   |       |
| 624                            |                        |   |       |
| 625                            |                        |   |       |
| 626                            |                        |   |       |
| 627                            |                        |   |       |
| 628                            |                        |   |       |
| 629                            |                        |   |       |
| 630                            |                        |   |       |
| 631                            |                        |   |       |
| 632                            |                        |   |       |
| 633                            |                        |   |       |
| 634                            |                        |   |       |
| 635                            |                        |   |       |
| 636                            |                        |   |       |
| 637                            |                        |   |       |
| 638                            |                        |   |       |
| 639                            |                        |   |       |
| 640                            |                        |   |       |
| 641                            |                        |   |       |
| 642                            |                        |   |       |
| 643                            |                        |   |       |



644 Table 2: Post hoc tests based on the B-GLMM for the comparisons of morning (AM) and  
 645 afternoon (PM) prevalence in temperate and tropical areas (corrected for multiple comparisons,  
 646 see text for details). Significant differences are in bold.  
 647

| Contrasts                         | estimate    | Standard error | Z ratio     | <i>P</i>           |
|-----------------------------------|-------------|----------------|-------------|--------------------|
| <b>temperate PM - AM</b>          | <b>3.70</b> | <b>0.42</b>    | <b>8.73</b> | <b>&lt; 0.0001</b> |
| tropical PM - AM                  | 0.37        | 0.34           | 1.10        | 0.32               |
| temperate AM - tropical AM        | -0.27       | 0.42           | -0.63       | 0.53               |
| <b>temperate PM - tropical PM</b> | <b>3.06</b> | <b>0.41</b>    | <b>7.45</b> | <b>&lt; 0.0001</b> |
| <b>temperate PM - tropical AM</b> | <b>3.43</b> | <b>0.42</b>    | <b>8.23</b> | <b>&lt; 0.0001</b> |
| tropical PM – temperate AM        | 0.64        | 0.42           | 1.51        | 0.20               |

648  
 649  
 650  
 651  
 652  
 653  
 654  
 655  
 656  
 657  
 658  
 659  
 660  
 661  
 662  
 663

664 **Legend to Figures:**

665 Figure 1: Location of the open area inside the Tropical Forest 1 - French Guyana, Nouragues  
666 French research, Saut-Pararé station (copyright: Florian Jeanne)

667

668 Figure 2: Prevalence of *Isospora* spp. (i.e. probability of infection, 1: infected, 0: not infected)  
669 in faecal samples from different bird species as a function of time elapsed through the day  
670 (standardized time of the day from 0 corresponding to sunrise to 1 corresponding to sunset) and  
671 of sampling site (Temperate Forest in black and Tropical Forest 1 + 2 in grey). Data points  
672 show actual prevalence, and predicted values fitted with the GLM model are in plain lines  
673 assorted with 95% confidence interval in light grey.

674

675 Figure 3: Parasite load of *Isopora* spp. (number of oocysts per gram of faeces) in different bird  
676 species as a function of time elapsed through the afternoon (standardized time of the day  
677 ranging from 0.5 corresponding to midday to 1 corresponding to sunset) and of sampling site  
678 (Temperate Forest in black and Tropical Forest 1 + 2 in grey). Data points show actual parasite  
679 loads, and predicted values fitted with the GLM model are in plain lines assorted with 95%  
680 confidence interval in light grey. Data presented here is restricted to infected birds sampled  
681 after a standardized hour of 0.5 (see text for more details).

682

683 Figure 4: Prevalence of *Isospora* spp. (probability of infection, 1: infected, 0: not infected) in  
684 faecal samples for different bird species as a function of time elapsed through the day  
685 (standardized time of the day from 0 corresponding to sunrise to 1 corresponding to sunset) and  
686 of sampling location within the Tropical Forest 1 site (open area in black, surrounding  
687 undergrowth in grey). Data points show actual prevalence (1: infected, 0: not infected), and

688 predicted values fitted with the GLM model are in plain lines assorted with 95% confidence  
689 interval in lighter grey.

690

691 Figure 5. Parasite load of *Isopora spp* (number of oocysts per gram of faeces) in different bird  
692 species as a function of time elapsed through the afternoon (standardized time of the day from  
693 0.5 corresponding to midday to 1 corresponding to sunset) and of sampling location within the  
694 Tropical Forest 1 site (open area in black and surrounding undergrowth in grey). Data points  
695 show actual parasite loads, and predicted values fitted with the GLM model are in plain lines  
696 assorted with 95% confidence interval in light grey. Data presented here is restricted to infected  
697 birds sampled after a standardized hour of 0.5 (see text for more details)

698

699 Figure 6. (a) Temperature (°C), (b) UV radiation index and (c) luminosity intensity (Lux) as a  
700 function of time elapsed through the day and of sampling location within Tropical Forest 1 site  
701 (open area in black and surrounding undergrowth in grey). At the time of sample collection,  
702 sunrise occurred at 06:00 am and sunset around 06:00pm. Data points show actual values, and  
703 predicted values fitted with the GLM model are in plain lines assorted with 95% confidence  
704 interval in light grey.

705

706

707

708

709

710

711

712