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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

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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 χ^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_{Temperate-Forest} [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_{Tropical-Forest} [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_{Tropical-}
212 Forest1[95%CI] = 0.43 [-1.18; 2.05] and slope_{Tropical-Forest2} [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 ± 15.95 *versus*
223 222.81 ± 46.01 , Tropical Forest 1: 29.71 ± 24.88 *versus* 91.06 ± 44.13 and Tropical Forest 2:
224 27.20 ± 23.25 *versus* 62.33 ± 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_{Temperate-Forest} [95%CI] = -0.002 [-3.01 ; 3.01]; mean \pm s.e. parasite load:
228 222.81 ± 46.01 oocysts per bird). In contrast, parasite load increased over time from 0 to around
229 200 oocyst per bird at sunset (slope_{Tropical-Forest} [95%CI] = 5.98 [2.27 ; 9.69], mean \pm s.e. parasite
230 load: 80.72 ± 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_{Tropical-}
235 Forest 1 [95%CI] = 6.18 [1.73 ; 10.6] and slope_{Tropical-Forest 2} [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_{openarea} [95% CI] = 8.87 [1.02 ;
242 16.72] versus slope_{undergrowth} [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 ± 441.20
248 oocysts/bird and forest undergrowth: 91.06 ± 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°C in open area and only around 30°C in the forest undergrowth between 12:00
253 and 15:00 (Gaussian GLM: time x area: $F_{1,94} = 4.88$, $P = 0.03$, time² 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: $\chi^2_1 = 56.39$, $P < 0.0001$; time²: $\chi^2_1 = 59.99$, $P < 0.0001$; location: $\chi^2_1 =$
259 169.08 , $P < 0.0001$, Fig. 6b and NB-GLM for luminosity: time: $\chi^2_1 = 52.01$, $P < 0.0001$; time²:
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 peppershike (*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 *Isospora* 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 *Isospora* 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 *Isospora* 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 *Isospora* 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; Hõrak *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 *Isospora* spp. parasite loads are to be compared,
359 then sampling time should be standardized at the same time of day.

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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

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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

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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,
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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]	
TEMPERATE FOREST - Souesmes	99 (14)	112 (95)	
Acrocephalidae			
<i>Hippolais polyglotta</i>	Melodious warbler	1 (1)	4 (4)
Aegithalidae			
<i>Aegithalos caudatus</i>	Long-tailed tit	1 (0)	4 (4)
Certhiidae			
<i>Certhia brachydactyla</i>	Short-toed tree creeper	4 (0)	2 (1)
Emberizidae			
<i>Emberiza citrinella</i>	Yellowhammer	0	1 (1)
Fringillidae	11 (1)	12 (10)	
<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
Locustellidae			
<i>Locustella naevia</i>	Common grasshopper-warbler	2 (0)	0
Motacillidae			
<i>Anthus trivialis</i>	Tree pipit	0	2 (2)
Muscicapidae	15 (1)	8 (8)	
<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)
Paridae	17 (5)	25 (17)	
<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)
Passeridae			
<i>Passer domesticus</i>	House sparrow	3 (0)	11 (11)
Phylloscopidae	10 (0)	9 (6)	
<i>Phylloscopus collybita</i>	Common chiffchaff	10 (0)	8 (6)
<i>Phylloscopus trochilus</i>	Willow warbler	0	1 (0)
Picidae			
<i>Jynx torquilla</i>	Eurasian wryneck	1 (0)	2 (2)
Prunellidae			
<i>Prunella modularis</i>	Dunnock	3 (1)	3 (1)
Sittidae	6 (1)	1 (1)	
<i>Sitta europaea</i>	Eurasian nuthatch	4 (1)	1 (1)

<i>Sturnus vulgaris</i>	Common starling	2 (0)	0
Sylviidae		24 (4)	26 (25)
<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)
Troglodytidae			
<i>Troglodytes troglodytes</i>	Eurasian wren	0	1 (1)
Turdidae			
<i>Turdus philomelos</i>	Song thrush	1 (0)	1 (1)
TROPICAL FOREST 1 - Pararé		79 (17)	74 (17)
Cardinalidae			
<i>Cyanocompsa cyanooides</i>	Blue-black grosbeak	1 (0)	0
Columbidae			
<i>Leptotila rufaxilla</i>	Grey-fronted dove	0	1 (0)
Cotingidae			
<i>Lipaugus vociferans</i>	Screaming piha	1 (0)	1 (0)
Formicariidae			
<i>Formicarius analis</i>	Black-faced antthrush	1 (0)	0
Furnariidae		14 (3)	14 (5)
<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)
Grallariidae			
<i>Hylopezus macularius</i>	Spotted antpitta	0	1 (0)
Pipridae		9 (1)	14 (0)
<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)
Poliptilidae			
<i>Ramphocaenus melanurus</i>	Long-billed gnatwren	1 (0)	0
Thamnophilidae		29 (8)	26 (8)
<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)
Thraupidae		4 (0)	0
<i>Tachyphonus surinamus surinamus</i>	Fulvous-crested tanager	2 (0)	0
<i>Lanio fulvus</i>	Fulvous shrike-tanager	2 (0)	0
Tityridae		5 (0)	3 (1)
<i>Onychorhynchus coronatus</i>	Royal Flycatcher	0	1 (0)
<i>Terenotriccus erythrurus</i>	Ruddy-tailed flycatcher	1 (0)	0
<i>Myiobius barbatus</i>	Sulphur-rumped flycatcher	4 (0)	2 (1)
Troglodytidae			
<i>Pheugopedius coraya</i>	Coraya wren	3 (1)	0
Turdidae			
<i>Turdus albicollis</i>	White-necked thrush	4 (1)	0
Tyrannidae		6 (2)	13 (3)
<i>Platyrrhynchus saturatus</i>	Cinamon-crested spadebill	0	1 (0)
<i>Platyrrhynchus 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)
Vireonidae			
<i>Hylophilus ochraceiceps</i>	Tawny-crowned greenlet	1 (1)	1 (0)
TROPICAL FOREST 1 OPEN AREA - Pararé		9 (0)	16 (10)
Fringillidae		0	4 (3)
<i>Euphonia cayennensis</i>	Golden-sided euphonia	0	1 (1)
<i>Euphonia violacea</i>	Violaceous euphonia	0	3 (2)
Thraupidae		6 (0)	9 (7)
<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)
Tyrannidae		2 (0)	2 (0)
<i>Pitangus sulphuratus</i>	Great kiskadee	1 (0)	0

<i>Tyrannus melancholicus</i>	Tropical kingbird	1 (0)	2 (0)
Vireonidae			
<i>Cyclarhis gujanensis</i>	Rufous-browed peppershrike	1 (0)	1 (0)
TROPICAL FOREST 2- Inselberg		49 (5)	40 (9)
Caprimulgidae			
<i>Caprimulgus nigrescens</i>	Blackish nightjar	0	1 (1)
Cardinalidae			
<i>Cyanocompsa cyanooides</i>	Blue-black grosbeak	1 (0)	0
Furnariidae		8 (3)	4 (4)
<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)
Galbulidae			
<i>Galbula albirostris</i>	Yellow-billed jacamar	1 (0)	0
Pipridae		17 (0)	10 (0)
<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)
Poliophtilidae			
<i>Microbates collaris</i>	Collared gnatwren	1 (0)	0
Thamnophilidae		12 (2)	15 (3)
<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)
Thraupidae		0	3 (0)
<i>Tachyphonus surinamus surinamus</i>	Fulvous-crested tanager	0	2 (0)
<i>Saltator grossus</i>	Slate-colored grosbeak	0	1 (0)
Tityridae		2 (0)	1 (0)
<i>Schoffornis turdina</i>	Brown-winged schiffornis	1 (0)	0
<i>Myiobius barbatus</i>	Sulphur-rumped flycatcher	1 (0)	1 (0)
Turdidae			
<i>Turdus albicollis</i>	White-necked thrush	4 (0)	1 (1)
Tyrannidae			
<i>Mionectes macconnelli</i>	McConnell's flycatcher	3 (0)	4 (0)

Vireonidae

Hylophilus ochraceiceps

Tawny-crowned greenlet

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1 (0)

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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>
temperate PM - AM	3.70	0.42	8.73	< 0.0001
tropical PM - AM	0.37	0.34	1.10	0.32
temperate AM - tropical AM	-0.27	0.42	-0.63	0.53
temperate PM - tropical PM	3.06	0.41	7.45	< 0.0001
temperate PM - tropical AM	3.43	0.42	8.23	< 0.0001
tropical PM – temperate AM	0.64	0.42	1.51	0.20

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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)

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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.

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