Artificial light at night amplifies seasonal relapse of haemosporidian parasites in a widespread songbird

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Abstract

Urban habitats can shape interactions between hosts and parasites by altering within-host processes such as resistance. Artificial light at night is common in urban environments, and chronic exposure can impair host immunity in ways that may increase infection. However, studies of causal links between this stressor, immunity, and infection dynamics are rare, particularly in migratory animals. Here, we experimentally tested how artificial light at night affects cellular immunity and haemosporidian parasite intensity across the annual cycle of migrant and resident subspecies of the dark-eyed junco (*Junco hyemalis*). We monitored an experimental group exposed to light at night and a control group under nighttime darkness as they passed through short days simulating early spring to longer days simulating the breeding season, followed by fall migration. Using generalized additive models, we show that artificial light at night increased inflammation, and leukocyte counts were greatest in early spring and fall. At the start of the experiment, few birds had active infections based on microscopy, but PCR revealed many birds had chronic infections. Artificial light at night increased parasitemia across the annual cycle, with strong peaks in spring and fall that were largely absent in control birds. As birds were kept in indoor aviaries to prevent vector exposure, this increased parasitemia indicates relapse of chronic infection during costly life history stages (i.e., reproduction). Although the immunological and parasitological time series were in phase for control birds, cross-correlation analyses also revealed artificial light at night desynchronized leukocyte profiles and parasitemia, which could suggest a general exaggerated inflammatory response. Our study shows how a common anthropogenic influence can shape within-host processes to affect infection dynamics.

Introduction

As suburban and urban habitats expand globally, certain species are attracted to these environments owing to a combination of altered microclimate, predation risk, and resource availability [1,2]. Such changes in biotic and abiotic conditions can have cascading and complex effects on wildlife [3,4]. When considering infectious disease, urban habitats can shape the interactions between hosts and parasites by altering within-host processes such as resistance or tolerance [5]. Understanding how urbanization affects these processes is especially important, as it can facilitate novel cross-species transmission opportunities between various host species [6].

One aspect of urban habitats that could have strong effects on these within-host processes is artificial light at night (ALAN). By altering animal circadian rhythms, ALAN can cause a suite of physiological changes relevant to infection [7]. In particular, chronic exposure to ALAN can suppress various aspects of host immune function, including cellular response and microbial killing ability [8,9]. Prolonged ambient light exposure can also dysregulate the inflammatory response, causing inflammation with pathological effects [10,11]. Ultimately, these changes likely alter host infection outcomes and parasite transmission [12]. In a rare test of causal relationships, ALAN altered gene regulatory networks and extended infectious periods for West Nile virus in house sparrows [13]. This suggests that ALAN could enhance the transmission of parasites to arthropod vectors and thus increase epidemic potential; however, the consequences of ALAN on infectious disease dynamics in wild hosts generally remain poorly understood.

Although the impacts of ALAN on immunity have been studied in various captive and free-living animals, these consequences have been less explored in migratory species. For birds in particular, many species migrate at night [14], facilitating exposure to light pollution along major migration routes [15]. Many birds also use suburban and urban habitats as stopover sites [16], and widespread ALAN in these environments can further affect habitat selection during migration [17,18]. Because physiological preparation for long-distance migration can itself suppress immune function [19,20], any effects of ALAN on within-host processes could be especially pronounced in migrants. If such changes amplify parasite transmission, urban habitats could become ecological traps for migratory species and in turn hotspots of spillover risk [21].

Here, we experimentally tested how ALAN exposure affects immunity and infection across the annual cycle of migratory and resident dark-eyed juncos (*Junco hyemalis*). Juncos are broadly distributed across North America [22], composed of subspecies that diverged 15,000

years ago and vary in migratory behavior, reproductive timing, and morphology [23]. Migratory slate-colored juncos (*J. h. hyemalis*) breed across Canada and the northern United States, migrating south in autumn. In the Appalachian Mountains, resident juncos (*J. h. carolinensis*) are sympatric with overwintering migrants from fall through early spring, when *J. h. hyemalis* initiate northern migration and *J. h. carolinensis* transition into breeding condition. To recreate the annual cycle of residents and migrants captured during sympatry, we modified photoperiod, which provides a consistent and predictive cue for animals to coordinate seasonal events such as reproduction and migration [24]. Mirroring natural changes in daylength across six months allowed us to capture these key life history events from early spring through late fall.

To determine effects of ALAN on within-host infection processes across these annual cycles in migrants and residents, we quantified cellular immunity and parasitemia. We focused on haemosporidians (i.e., causative agents of avian malaria) [25], which are common in juncos and are transmitted by arthropod vectors from spring through autumn [26,27]. Haemosporidians cause a short, acute infection followed by a chronic phase characterized by low parasitemia and latency in peripheral host tissue [25,28]. Parasites present in hosts sampled in winter therefore signal chronic infections [27,29]. However, seasonal relapse of chronic infections commonly occurs from physiological changes in preparation for bird reproduction or migration [29–31]. Manipulating photoperiod in an indoor aviary free of potential vectors allowed us to isolate effects of ALAN and seasonality on within-host processes, whereas field comparisons may also have greater exposure to vectors in urban habitats [32]. We predicted ALAN would promote inflammation and hematological indices of physiological stress (i.e., ratios of heterophils to lymphocytes [33]) and that immune dysregulation could cause chronic infections to reactivate. Because stages of the annual cycle carry distinct energetic costs, we also expected these relationships would vary by photoperiod and migratory strategy. Parasitemia could be greatest under ALAN in late spring, when migrants prepare for northern migration and residents start summer breeding. However, migrants could display greater parasitemia than residents in early autumn, when residents complete breeding while migrants initiate their southern fall migration.

Methods

Junco sampling and housing

96 In December 2017, we captured adult male migrant and resident juncos at the Mountain Lake
97 Biological Station (Pembroke, VA). Each bird was fitted with an aluminum U.S. Fish & Wildlife
98 Service leg band. Subspecies were differentiated by bill color, plumage, and size [22]. Residents
99 (n = 18) and migrants (n = 24) were housed with *ad libitum* food and water in an indoor aviary at
100 Indiana University in Bloomington, Indiana. During a prior experiment, we gradually increased
101 then decreased photoperiod to simulate a full annual cycle through 2018 [34], and birds were
102 maintained in a photosensitive state at 9 hours daylength (i.e., 9L) until our experiment in 2019.
103

104 *Experimental design*

105 We randomly housed equal numbers of residents and migrants in mixed-flock, free-flying rooms
106 in two separate treatments. Starting in February 2019, we simulated another full annual
107 photoperiodic cycle while exposing control birds under natural day lengths to total nighttime
108 darkness (ND) and treatment birds to constant ~2.5 ± 0.5 lux average intensity during night
109 (ALAN). ALAN intensity was determined from a dose-dependent study in great tits, in which
110 0.5–5 lux increased reproductive activity [35], and such levels reflect ALAN exposure of urban
111 birds [36]. All rooms blocked natural sunlight and provided photoperiod light via floorlamps. We
112 increased photoperiod every fourteen days by 0.3–1 hour from February 16 to September 2
113 (9L:15D to 16L:8D), continued for eight weeks at 16L:8D, and then decreased photoperiod until
114 November 22 (16L:8D to 9L:15D). At each timepoint, we captured free-flying indoor birds 30
115 minutes after the onset of morning photoperiod light. For a complementary study of reproductive
116 physiology, birds received a standardized intramuscular challenge of gonadotropin releasing
117 hormone at each timepoint [37]. Thirty minutes after injection, we collected small blood samples
118 using sterile 26G needles and made thin blood smears for hematology. Birds were kept in opaque
119 bags between injections to reduce stress, and the 30 minutes of holding time between challenge
120 and bleeding was uniform across birds and is unlikely to affect circulating leukocyte counts [33].
121 We collected repeated blood smears across nine timepoints (Table S1). Some birds were
122 removed from the study due to injury or mortality, resulting in 21 migrants and 12 residents for
123 analyses.
124

125 *Hematology and PCR*

126 Blood smears were stained with buffered Wright–Geimsa (Astral Diagnostics Quick III). From
127 each sample (n = 297), we evaluated leukocyte profiles and parasite intensity using light
128 microscopy (AmScope, B120C-E1). A single observer, blind to the treatment and migratory
129 strategy of each bird, first recorded the number of white blood cells under 40X magnification
130 across 10 random fields to estimate inflammatory state and investment in cellular immunity.
131 Next, a differential count was performed by recording the identity of the first 100 leukocytes at
132 1000X magnification [38]. Approximately 100 fields of view at 1000X magnification were
133 screened for *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* [25]. We derived the mean total
134 white blood cell count (total leukocytes), ratio of heterophils to lymphocytes (HL ratios), and
135 total number of haemosporidian parasites (parasite intensity) as three separate response variables.

136 As microscopy primarily detects active haemosporidian infection [25,28], we used PCR
137 to detect chronic infections at the start of our experiment (i.e., 9L). We extracted genomic DNA
138 from erythrocytes in Longmire's solution using RSC Whole Blood DNA Kits (Promega) run on
139 a Maxwell RSC at Indiana University, and DNA was eluted in 70 µL buffer. We used previously
140 published primers and PCR protocols to amplify a conserved fragment of the haemosporidian
141 mitochondrial rRNA gene common to *Plasmodium*, *Haemoproteus*, and *Leucocytozoon [39,40]*.
142 We re-screened any negative birds after initial PCR to reduce possible risks of false negatives.

143

144 *Statistical analysis*

145 We analyzed all response variables using R [41]. We first used generalized additive mixed
146 models (GAMMs) with the *mgcv* package to account for potential nonlinear effects of
147 seasonality (i.e., photoperiod) and treatment [42]. We analyzed data from migrants and residents
148 separately, as initial analyses suggested no difference in any of these three outcomes by
149 migratory strategy or its interaction with treatment (Table S2). For each response variable per
150 subspecies, we fit a GAMM with treatment, a smoothed effect of photoperiod, and their
151 interaction using thin plate splines [43]. GAMMs included a random effect of individual band
152 number to account for repeated measures. We modeled both total leukocytes and HL ratios with
153 Tweedie distributions, whereas we used a zero-inflated Poisson distribution for parasitemia.

154 To test temporal dependence between cellular immunity and infection, we estimated the
155 cross-correlation between log-transformed parasitemia and (*i*) log-transformed total leukocytes
156 and (*ii*) square-root HL ratios for all photoperiod time lags between each time series [44]. We
157 estimated cross-correlation coefficients for each treatment within migrants and residents. We
158 then fit an additional set of GAMs with a smoothed effect of photoperiod lag, treatment, and
159 their interaction with cyclic cubic splines and a Gaussian distribution for each strata of the data.

160

161 **Results**

162 *ALAN effects on leukocyte profiles*

163 ALAN increased total leukocytes compared to ND birds (Fig. 1), and effect sizes were similar
164 for residents and migrants (Table 1). Regardless of migratory strategy, we also found strong
165 seasonal (i.e., photoperiodic) variation in total leukocytes, with greater inflammation at the start
166 and end of our experiment (i.e., early spring and late fall). Although GAMMs explained up to
167 59% of the deviance in total leukocytes, seasonal patterns did not vary by treatment. In contrast,
168 we found weaker effects of treatment and photoperiod on HL ratios (Table S3). ALAN did not
169 broadly affect HL ratios (Fig. 1). Only migrants displayed an effect of photoperiod on HL ratios,
170 for which this measure of physiological stress was highest in ALAN birds during early spring.

171

172 *Haemosporidian intensity under ALAN*

173 At our first blood smear collection (10L), the prevalence of active infection (i.e., infected
174 erythrocytes detectable with microscopy) was on average 14% between experimental groups;
175 only ALAN residents had no microscopy-detectable infection (Fig. S1). PCR confirmed at least
176 50% of birds per treatment had chronic infections at the start of the study (i.e., 9L; Fig. S2), and
177 the odds of PCR-detectable infection did not differ by treatment or subspecies (Table S4). Most
178 PCR-negative birds at 9L did not show microscopically detectable infection at our first blood
179 smear collection (10L, 92%; Fig. S3). Diagnostic mismatches suggest PCR-negative birds likely
180 harbored latent infections (i.e., those present in peripheral tissue) that relapsed later in the

experiment, although false negatives are also possible [25,45]. However, by the end of the study, active infections were common in all groups, ranging from 30% to 100% ($\bar{x}$ = 63%; Fig. S1).

Our GAMMs revealed marked effects of ALAN on seasonal parasitemia (Fig. 1). Haemosporidian intensity was higher under ALAN compared to birds held under nighttime darkness, although this effect was greater in resident juncos (Table 2). For all ALAN birds, parasitemia peaked around 12L, corresponding to spring, followed by a secondary but smaller parasite intensity peak in photoperiods corresponding to autumn (i.e., 12–10L). Control residents had relatively minor seasonal changes in parasitemia, whereas control migrants also increased haemosporidian intensity in photoperiods corresponding to both spring and fall (Fig. 1).

Cross-correlation between immunity and infection

ALAN affected the cross-correlation between cellular immunity measures and haemosporidian intensity across our experiment (Tables S5 and S6). For control residents, correlations between total leukocytes and parasitemia showed strong nonlinearity and were most positive near the zero-photoperiod lag, whereas HL ratios had weak effects on parasitemia (Fig. 2). However, ALAN removed this synchrony, resulting in positive correlations between total leukocytes and haemosporidian intensity across all lags and a non-significant trend. This desynchronization of immunity–parasitemia relationships with ALAN was similar for migrants, but ND birds instead showed negative correlations between total leukocytes and parasitemia and positive correlations between HL ratios and parasitemia when the experimental time series were in phase (Fig. 2).

Discussion

Understanding how anthropogenic habitats affect host–parasite interactions is important to forecast how these environments will shape infection risks for wildlife and humans. Here, we experimentally showed that ALAN, a pervasive component of suburban and urban habitats, increased total leukocytes and haemosporidian intensity in resident and migratory songbirds. Treatment birds had pronounced parasitemia peaks in the spring and fall that were largely absent in control birds, indicating that ALAN exposure caused relapse of chronic infection during

209 energetically costly life history stages. Additionally, although our immunological and
210 parasitological time series were in phase for control birds, ALAN desynchronized leukocyte
211 profiles and haemosporidian intensity, which could suggest general immune dysregulation.

212 Because our study occurred in an indoor aviary and PCR confirmed many birds had
213 chronic infections, elevated total leukocytes and haemosporidian intensity in birds exposed to
214 ALAN reflect altered within-host infection dynamics rather than acute infections resulting from
215 vector exposure [32]. In some contexts, ALAN can be immunosuppressive [8,9]; however, the
216 elevated leukocyte counts observed here more likely indicate inflammation in response to
217 haemosporidians [46]. In mice, ALAN can induce an exaggerated inflammatory response driven
218 by proinflammatory cytokines, excessive production of which causes immunopathology and can
219 limit control of infection [10,11]. The mechanisms by which the avian immune system controls
220 haemosporidians remains unclear [47], but adaptive responses are likely also important [48,49].
221 Related to a possibly exaggerated inflammatory response, total leukocytes were broadly
222 positively associated with parasite intensity regardless of time lag, whereas control birds showed
223 more acute synchrony with parasitemia. Such results suggest that ALAN could cause immune
224 dysregulation, although quantification of immune gene expression would help understand the
225 specific pathways by which ALAN affects the reactivation of haemosporidian infection [50].

226 Although our results agree with the prediction that ALAN would promote inflammation,
227 we observed generally weak effects on HL ratios. HL ratios can signal elevated glucocorticoids
228 in response to stressors [33], although increases occur more slowly than plasma corticosterone
229 and can be caused by not only intense stressors but also more mild and chronic challenges [51].
230 Accordingly, our results suggest chronic stimulation of the hypothalamic–pituitary–adrenal axis
231 may not be the underlying mechanism linking ALAN with parasite relapse. This finding is
232 similar to other work on ALAN and within-host processes (e.g., infectious periods for West Nile
233 virus in house sparrows), which suggested relationships between ALAN and increased viremia
234 were not mediated by corticosterone [13]. Alternatively, relationships between ALAN,
235 immunity, and parasitemia could be mediated by other seasonally varying hormones such as
236 testosterone or melatonin [52]. In particular, melatonin is secreted for a longer duration in short
237 days and can facilitate immunostimulation [53]. Fluctuations in host melatonin can also affect
238 life cycle periodicity of some haemosporidians [25]. Suppression of melatonin with ALAN could

239 explain seasonal relapse during periods when immunity would otherwise be enhanced [54].
240 Future work could experimentally assess the hormonal basis of ALAN-induced parasite relapse.

241 Residents and migrants generally responded in a similar fashion to ALAN, with elevated
242 leukocytes and increased parasitemia in spring and fall. However, both control and treatment
243 migrants displayed reactivation of chronic infections in spring, likely signalling physiological
244 changes in preparation for migration combined with those for reproduction [29,30]. In contrast,
245 whereas residents exposed to ALAN also experienced dramatic spring relapse, control birds
246 showed only weak increases in parasitemia in late autumn. These patterns suggest that the
247 effects of ALAN on parasite relapse may be greater during periods of reproduction rather than
248 preparation for migration. Mounting an immune response against haemosporidians can be costly,
249 which could drive weaker parasite control during energetic trade-offs with reproductive activity
250 [47,55]. However, our study only assessed transitions into migratory preparedness and not long-
251 distance migration itself, which could impose stronger effects on parasite control and therefore
252 on cycles of latency and reactivation [56,57]. Lastly, fall increases in parasitemia for both
253 residents and migrants under ALAN could again signal melatonin suppression [52,54].

254 Although the mechanisms linking ALAN with parasite relapse require further study,
255 particularly for non-migratory hosts, our findings also have implications for the population-level
256 consequences of infection. For haemosporidian parasites in particular, fitness costs for birds can
257 be highly variable [25]. In some cases, haemosporidians have caused dramatic population
258 declines in hosts such as Hawaiian honeycreepers and possibly house sparrows in Europe
259 [58,59]. Relapse of chronic infections under ALAN in anthropogenic habitats could accordingly
260 either facilitate infection-related population declines in less tolerant avian hosts or increase
261 parasitemia in tolerant species that instead serve as sources of infection for generalist vectors,
262 thereby increasing transmission in the local avian community. To reduce such negative impacts,
263 alternative lighting schemes in suburban and urban environments, such as warmer light spectra
264 or restricting the use of broad-spectrum light during demanding stages of avian annual cycles
265 (e.g., migration), warrant further investigation as ecologically friendly interventions [60,61].

266 More generally, our study highlights reactivation of chronic or latent infection as a novel
267 mechanism by which urbanization can affect infectious disease dynamics. Examining how
268 ALAN affects cycles of latency and reactivation in other host and parasite systems, such as

Borrelia burgdorferi and arboviruses in songbirds [62,63] or henipaviruses and herpesviruses in bats [64,65], could inform diseases risks relevant for wildlife conservation, domestic animal health, and human health. Such work could be especially informative for synanthropic wildlife and migratory species that are increasingly becoming sedentary in anthropogenic habitats [66].

Author contributions

DS, DJB, and EDK designed the study; DS, DJB, JDM, KMT, and SW collected samples; QP analyzed blood smears; KMT conducted PCR; and DJB analyzed data and wrote the manuscript. All authors contributed to revisions.

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Ethics

All procedures were approved by the Indiana University Institutional Animal Care and Use Committee (18-030) and conducted under permits issued by the Virginia Department of Game and Inland Fisheries (052971) and U.S. Fish and Wildlife Service (20261).

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Data availability

Data are available in the Dryad Digital Repository:
https://datadryad.org/stash/share/IxJYbB61kJJifIIXuEC72ZcIPEpblW6TjIII6sQ8rQM

298 **Tables**

299 Table 1. Effects of ALAN treatment, photoperiod, and their interaction on total leukocytes.
300 GAMMs were stratified by migratory strategy and fit using restricted maximum likelihood.
301 Fixed effects are presented with model coefficients (categorical) or the estimated degrees of
302 freedom (EDF) and test statistics. Deviance explained (%) is presented for both GAMMs.

	Residents (deviance explained=52%)					**Migrants (deviance explained=59%)**				
Term	**β**	***t***	**EDF**	***F***	***p***	**β**	***t***	**EDF**	***F***	***p***
Intercept	-0.30	-1.88			0.06	-0.28	-2.51			0.01
ALAN	0.55	2.67			0.01	0.50	3.18			<0.01
s(photoperiod)			3.27	5.32	<0.01			2.90	7.03	<0.01
s(photoperiod, ND)			0.50	0.34	0.60			0.00	0.07	0.99
s(photoperiod, ALAN)			1.00	2.34	0.13			3.13	2.96	0.07

303

304 Table 2. Effects of ALAN treatment, photoperiod, and their interaction on parasite intensity.
305 GAMMs were stratified by migratory strategy and fit using restricted maximum likelihood.
306 Fixed effects are presented with model coefficients (categorical) or the estimated degrees of
307 freedom (EDF) and test statistics. Deviance explained (%) is presented for both GAMMs.

	Residents (deviance explained=63%)					**Migrants (deviance explained=54%)**				
Term	**β**	***t***	**EDF**	***F***	***p***	**β**	***t***	**EDF**	***F***	***p***
Intercept	-5.14	-2.47			0.01	0.04	0.11			0.91
ALAN	6.90	3.27			<0.01	2.12	3.92			<0.01
s(photoperiod)			1.00	1.05	0.31			2.12	10.3	0.01
s(photoperiod, ND)			1.92	17.2	<0.01			2.33	10.2	0.01
s(photoperiod, ALAN)			3.98	276	<0.01			3.18	20.7	<0.01

308

Figures

Figure 1. Effects of ALAN on total leukocytes, HL ratios, and haemosporidian intensity across the simulated annual cycle (i.e., photoperiod, L) of resident and migrant dark-eyed juncos. Points show medians with jackknife estimates of standard error and are jittered to reduce overlap. Lines and shaded bands show the fitted values and 95% confidence interval from each GAMM. The vertical axes are displayed with a modulus transformation to accommodate lower bounds at zero.

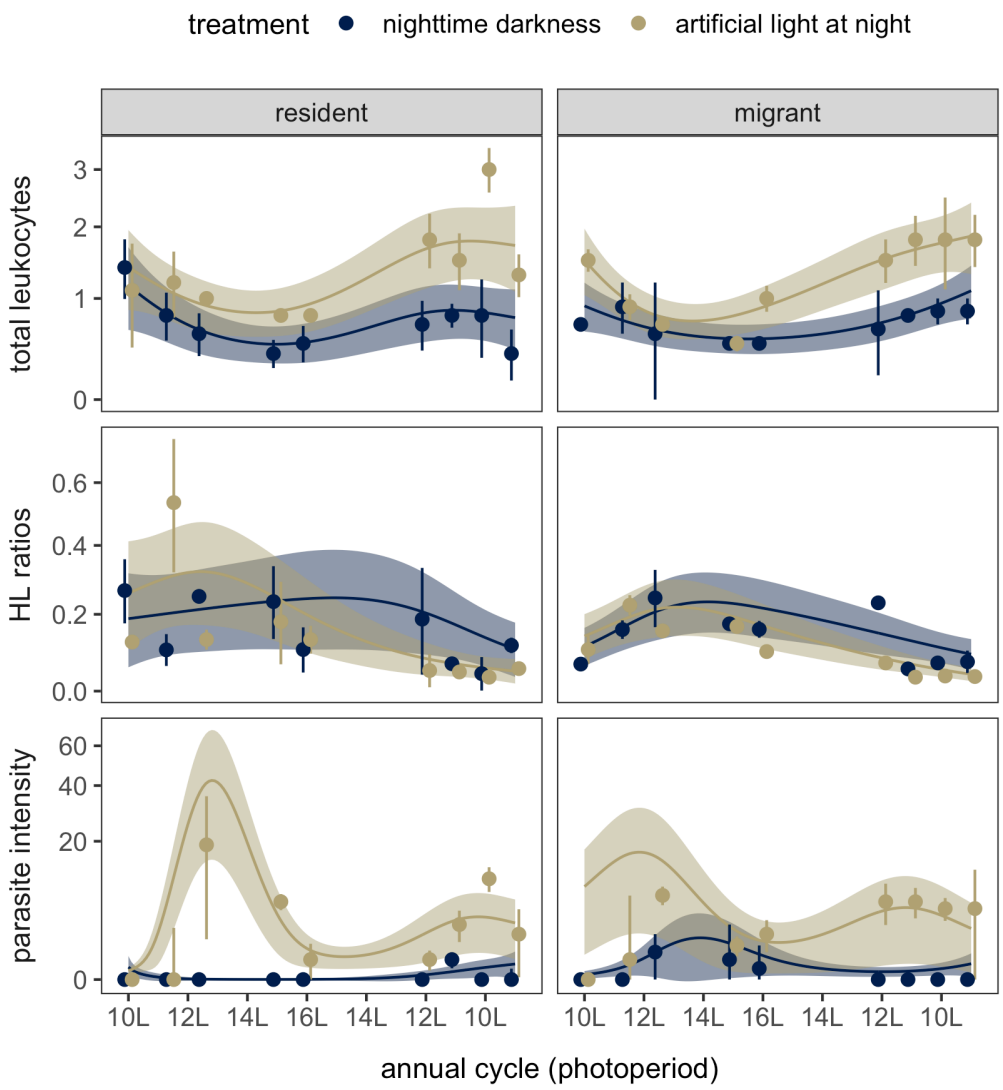
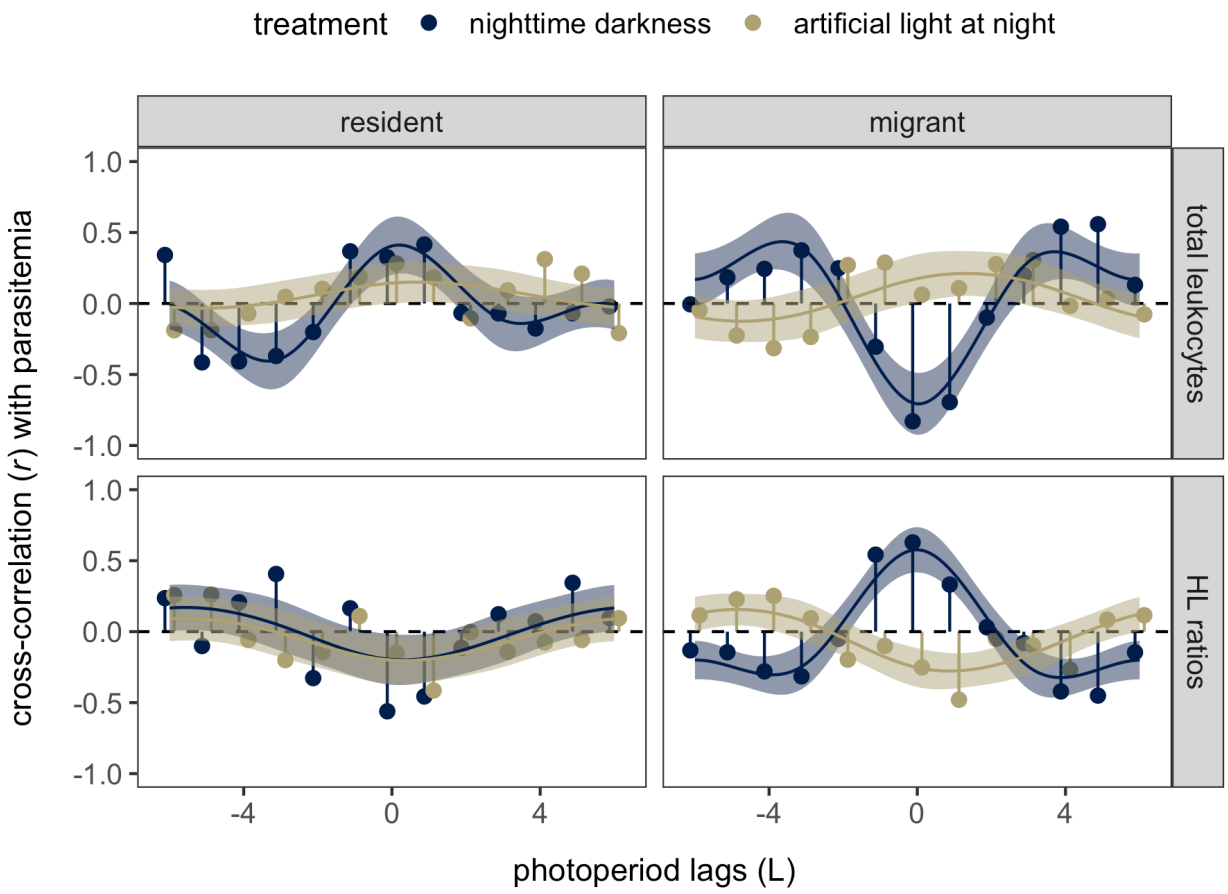


Figure 2. Cross-correlation between immune measures (total leukocytes and HL ratios) and haemosporidian intensity throughout the duration of the experiment. Points display correlation coefficients (*r*) at each photoperiod lag and migratory strategy and are colored by treatment. Lines and shaded bands display the fitted values and 95% confidence interval from each GAM.



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