

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/164876/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Arapi, Elissavet A., Reynolds, Mike , Ellison, Amy R. and Cable, Jo 2023. Restless nights when sick: ectoparasite infections alter rest-activity cycles of diurnal fish hosts. *Parasitology* 10.1017/S0031182023001324

Publishers page: <https://doi.org/10.1017/S0031182023001324>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



1 **Restless nights when sick: ectoparasite infections alter rest-activity cycles of diurnal**
2 **fish hosts**

3

4 Elissavet A Arapi¹, Mike Reynolds¹, Amy R Ellison^{1,2}, Jo Cable¹

5

6 ¹School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK

7 ²School of Natural Sciences, Bangor University, Bangor, LL57 2DG, UK

8

9 Author for correspondence: Arapi Elissavet, E-mail: ArapiE@cardiff.ac.uk

10 **Abstract**

11 Circadian rhythms are timekeeping mechanisms responsible for an array of biological
12 processes. Disruption of such cycles can detrimentally affect animal health. Circadian rhythms
13 are critical in the co-evolution of host-parasite systems, as synchronization of parasite rhythms
14 to the host can influence infection dynamics and transmission potential. This study examines
15 the circadian rhythms in behaviour and activity of a model fish species (*Poecilia reticulata*) in
16 isolation and in shoals, both when uninfected and infected with an ectoparasite (*Gyrodactylus*
17 *turnbulli*). Additionally, the rhythmical variance of parasite activity under different light
18 conditions as well as rhythmical variance in parasite transmissibility was explored. Overall,
19 infection alters the circadian rhythm of fish, causing nocturnal restlessness. Increased activity
20 of gyrodactylids on the host's skin at night could potentially contribute to this elevated host
21 activity. Whilst migration of gyrodactylids across the host's skin may have caused irritation to
22 the host resulting in nocturnal restlessness, the disruption in guppy activity rhythm caused by
23 the expression of host innate immunity cannot be excluded. We discuss the wider
24 repercussions such behavioural responses to infection have for host health, the implications
25 for animal behaviour studies of diurnal species as well as the application of chronotherapeutic
26 approaches to aquaculture.

27 **Keywords:** Circadian rhythms, nocturnal activity, rest deprivation, infection, disease
28 susceptibility, host health

29

30 **Key Findings**

- 31 • Ectoparasites alter daily rhythmic activity of their hosts, resulting in greater nocturnal
32 restlessness both individually and in shoals.
- 33 • Circadian rhythmicity in activity was present and distinctly different between uninfected
34 and infected fish.
- 35 • Peaks in activity may be driven by adaptive immune responses to infection such as
36 infection resolution and repair, which are elevated at night.
- 37 • Parasite behaviour does not exhibit a daily rhythmical variance, but peaks in the dark,
38 coinciding with infected fish behaviour.
- 39 • The use and application of chronotherapy to maximize treatment efficacy could be a
40 potential solution to the problem of infectious diseases.

41 **Introduction**

42 Circadian rhythms are intrinsic timekeeping mechanisms responsible for the cyclic repetition
43 of metabolic, behavioural and psychological processes in all living organisms, typically over a
44 24-hour period (Liang et al., 2015; Sollars and Pickard, 2015). They are endogenously
45 generated by self-sustaining biological clocks, encoded by “clock genes”, and entrained by
46 environmental cues such as light and temperature (Piggins, 2002). Their disruption can affect
47 an array of biological activities such as rest-activity cycles, immunity and disease susceptibility
48 (Bass and Lazar, 2016), as shown in humans if natural circadian cues are ignored due to shift
49 work, jet-lag and general sleep deprivation (Takahashi et al., 2008).

50 Sleep is a complex enigma that serves multiple functions (Krueger et al., 2016), most
51 notably provisioning critical restorative and repair processes (Adam, 1980; Benington and
52 Craig Heller, 1995; Cirelli and Tononi, 2008; Helvig et al., 2016). The general assumption that
53 all species ‘sleep’ is controversial, with some animals entering a restful state that does not
54 necessarily fulfil descriptors depicting true sleep (Siegel, 2008). Recent evidence of true sleep
55 (including Rapid Eye Movement sleep phase; REM), however, has now been reported in
56 zebrafish (Leung et al., 2019). Furthermore, a consistent observation across taxa is that

57 disturbances to 'rest-activity' cycles, and thus disruption of circadian rhythms, can have
58 detrimental consequences for health with respect to disease, even reducing life expectancy
59 (Kripke et al., 2002; Davidson, 2006).

60 In fish, circadian rhythms govern biological activities ranging from reproduction,
61 spawning, smoltification and maturation to immune responses. Circadian rhythms have been
62 observed in activity patterns of various fish of economic importance including the golden shiner
63 (*Notemigonus crysoleucas*), goldfish (*Carassius auratus*), lake chub (*Couesius plumbeus*),
64 Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) (see Reeb, 2002).
65 In aquaculture, manipulating photoperiods, such as extending the light period in diurnal
66 species, can improve rearing quality and promote increased growth rates (Boeuf and Le Bail,
67 1999). In the extreme, constant light is used to improve feed utilisation (Boeuf and Le Bail,
68 1999) or control maturation and puberty (Taranger et al., 2010). However, this may have
69 negative implications for health and disease resistance, as immune functions are often highly
70 rhythmic, enabling organisms to mount their most efficient response at times when risk of
71 infection or injury is highest (Ellison et al., 2021). Conversely, immune factors and infections
72 can affect expression of molecular clocks (Adams et al., 2013; Castanon-Cervantes et al.,
73 2010). So, disruption of normal circadian cycles can impact immune responses and may
74 increase disease risks (Ellison et al., 2021). Given the increased understanding of the intricate
75 link between fish body clocks and their immune systems, harnessing knowledge of circadian
76 rhythms may be hugely beneficial against infectious diseases. However, for chronobiological
77 approaches to tackle infectious diseases, rhythms of both the fish and their associated
78 parasites must be considered.

79 Parasites can directly impact host rest-activity cycles (Ibarra-Coronado et al., 2015),
80 which are associated with activation of immune defences (Preston et al., 2009). Moreover,
81 individuals are most at risk of acquiring parasitic infections when sleep deprived (Bryant et al.,
82 2004; Majde and Krueger, 2005). Thus, the reciprocal interaction between rest-activity cycles
83 and immune functioning is complex (Opp, 2009). The underlying mechanism appears to be
84 stress-related, which in turn affects the immune system, causing increased susceptibility to

85 infection and subsequently higher mortality rates (Davidson, 2006; Penev et al., 1998). In fish,
86 immune responses to infectious diseases have been extensively studied in the past. Now,
87 increasingly more studies investigate the disruption of fish circadian rhythms by parasites, as
88 in the case of zebrafish (*Danio rerio*), where established *Pseudoloma neurophilia* infections
89 induced major transcriptional changes in the host brain (Midtunn et al., 2020). However, little
90 is known about how parasites might alter fish resting periods and the long-term implications
91 of disrupted circadian rhythms.

92 Parasite circadian rhythms are critical in the co-evolution of host-parasite systems, as
93 synchronization of their rhythms can influence infection dynamics and transmission potential
94 (O'Donnell et al., 2011). Parasite circadian rhythms are apparent in oviposition (*Schistosoma*
95 *haematobium* see Theron and Combes, 1995; *Passalarus ambiguous* see Rinaldi et al., 2007),
96 timing of asexual reproduction (*Plasmodium chabaudi* see Mideo et al., 2013) as well as
97 expression of certain metabolism genes (*Trypanosoma brucei* see Rijo-Ferreira et al., 2017).
98 Circadian rhythms have also been implicated in detachment of parasites from their host
99 (Doube, 1975), as well as host immune evasion by secretion of chemical signals (DuRant et
100 al., 2015). For monogenean ectoparasites, rhythmical variance has been observed in egg
101 laying and hatching (*Discocotyle sagittata* see Gannicott and Tinsley, 1997; *Entobdella soleae*
102 see Kearns, 1967; *Benedenia ladjani* see Ernst and Whittington, 1996). With circadian rhythms
103 seemingly affecting various aspects of a parasite's life cycle, the impact of circadian rhythms
104 on infection potential and dynamics needs to be further explored.

105 One of the most ubiquitous groups of monogenean parasites infecting teleost fish are
106 the gyrodactylids. These parasites are known to infect multiple fish of aquacultural importance,
107 including cyprinids (Zietara and Lumme, 2002) and salmonids (Harris et al., 2004), and can
108 have a major economic impact on the industry. Little is known regarding daily activity rhythms
109 of gyrodactylids, such as movement on the host and host-seeking behaviour, with the
110 exception of one study which reported greater variation of *in vitro* parasite activity in dark
111 compared to light conditions (Brooker et al., 2011). From a host perspective, sticklebacks

112 (*Gasterosteus aculeatus*) were more susceptible to *Gyrodactylus gasterostei* when exposed
113 to prolonged photoperiods; due to changes in host physiology, condition and immune
114 responses (Whiting et al., 2020). However, whether gyrodactylids exhibit a light-dependant
115 behaviour or parasite activity has true circadian rhythmicity has yet to be studied.

116 The current study investigates (a) the impact of an ectoparasitic infection on host rest-
117 activity cycles, and (b) the rhythmical variance in parasite activity and behaviour. For this
118 study, we used the tropical Trinidadian guppy (*Poecilia reticulata*)-*Gyrodactylus turnbulli*
119 model; a system that has been subject to extensive epidemiological and behavioural
120 investigations (Bakke et al., 2007). Although this parasite has been known to cause
121 behavioural modifications in its typically diurnal host (e.g., foraging and swimming
122 performance; Cable et al., 2002; Kolluru et al., 2009), the daily dynamics of guppy-gyrodactylid
123 interactions have, until now, been overlooked. Therefore, we are exploring the behaviour of
124 infected hosts compared to their uninfected conspecifics both when isolated and in shoals and
125 we discuss the implications that this may have for host health and aquaculture in general.

126 **Materials and methods**

127 *Host and parasite origins and maintenance*

128 Trinidadian guppies (*Poecilia reticulata*) originating from the Lower Aripo River, Trinidad (wild-
129 type strain), or from a commercial wholesaler (ornamental strain) were transported to Cardiff
130 University Aquarium. Fish stocks were housed separate in 70 L tanks of dechlorinated water
131 (approx. 1 fish/ 1L water, as recommended by OATA; Ornamental Aquatic Trade Association),
132 in 24 ± 1 °C in a 12:12 h light: dark regime and fed daily with Aquarian® tropical fish flakes
133 supplemented with live *Daphnia magna* and freshly hatched *Artemia* nauplii. For all
134 experiments, female or juvenile guppies were used and size-matched to avoid size
135 variability, which is known to affect parasite load (Cable and van Oosterhout, 2007). For
136 each experiment, only one fish stock and single sex fish were used, to avoid confounding
137 variables.

138 For experimental infections, the *Gt3* strain of *Gyrodactylus turnbulli* was used; isolated in
139 1997 from, and subsequently maintained on ornamental guppies (as in Stewart et al., 2017).
140 For all experimental infections, a sacrificed donor was placed close to a recipient fish
141 anaesthetised with 0.2% tricaine methanesulfonate (MS222). Direct contact between hosts
142 facilitated transfer of gyrodactylids, as observed under a dissecting microscope with fibre optic
143 illumination. Fish were infected with 30 parasites each, representative of burdens reached
144 after 5 days for an individually isolated fish experimentally infected with two worms on Day 0
145 (e.g., van Oosterhout et al., 2003).

146 *Experimental design*

147 Overall, four experiments were performed: two compared the rhythmical activity of the guppy
148 host when uninfected and infected with *Gyrodactylus turnbulli* and two explored the
149 rhythmical variance in activity of the actual parasite. For all experiments, we report the
150 Zeitgeber Time (ZT) system, where ZT is a unit of time based on light Zeitgeber. The ZT
151 denotes when the lights go on and off, in this case, ZT0 (07:00 h) denotes lights on and ZT12
152 (19:00 h) lights off (Karatsoreos and Silver, 2017). There was no light fade to simulate
153 sunrise/sunset conditions. For nocturnal observations, infrared lights (light intensity 1.2-1.3
154 Lux; Precision Gold Digital Light Meter) were used compared to the white light (500 Lux
155 intensity) used during the day, as infrared illumination is invisible to the animals being
156 observed but visible to infrared cameras (Widder et al., 2005).

157 For all experiments, uninfected control fish were sham infected to account for handling
158 time and then returned to 1 L dechlorinated water pots to recover. No anaesthetic associated
159 mortalities occurred during this study and the anaesthesia process, with a 0.02% MS222 dose,
160 seemed to have no effect on host and parasite behaviour and survival (Cambel et al., 2015).
161 Following infection (and sham infection), fish were transferred to the experimental tank in a
162 small glass dish containing dechlorinated water, ensuring the fish was never out of water nor
163 was there any risk of nets dislodging the ectoparasites. Once all experimental trials concluded,
164 infected fish were treated with an anti-helminthic drug, 0.1% Levamisole, to eliminate any

165 parasites and then screened clear under the microscope three consecutive times to ensure
166 that they were parasite-free (Schelkle et al., 2009).

167 *Automated monitoring of host behaviour*

168 Behavioural arrays used in Experiment 1 for monitoring infected and uninfected individual fish
169 consisted of 3 acrylic tanks (22 cm length x 10 cm width x 20 cm depth; Figure 1), positioned
170 within two rows of TriKinetics behavioural monitors. Each behavioural array tank was filled
171 with 1.25 L of dechlorinated water and white card paper on each side of the tanks avoided any
172 external disturbances to the fish. Ten infrared beams passed through each tank, five from the
173 top monitor and five from the bottom monitor, from the infrared emitters to the receivers. The
174 monitors were connected to the TriKinetics software, which automatically recorded how many
175 times a fish passed through a beam within a certain time period.

176 *Experiment 1: Impact of infection on daily activity of isolated guppies*

177 To observe whether there is a difference in activity between uninfected and infected isolated
178 wild-type guppies under a 12:12 h light: dark regime, female adult guppies were size-matched
179 (15.68 ± 0.95 mm) and two experimental groups were created: uninfected controls ($n = 11$)
180 and infected experimental fish ($n = 10$). Fish remained in individual 1 L containers for 7 days
181 prior to start of the experiment. On Day 1, experimental guppies were infected with exactly 30
182 gyrodactylids, whilst control fish were sham infected to control for handling time. Each fish was
183 then placed into a 1 L dechlorinated pot to recover, before being transferred to a behavioural
184 array tank for acclimation. At 07:00, the following day (Day 2), the arrays started monitoring
185 guppy activity every minute for 48 h. On Day 4, fish were removed from the tanks,
186 anaesthetised and screen under the microscope. The experimental fish were screened to
187 record their final parasite load (mean intensity 73, range 49 - 93) and the control fish were
188 screened in order to ensure that no contamination had occurred, with control fish indeed
189 remaining parasite free. Fish activity was recorded as the counts of infrared beam breaks per
190 tank, as retrieved from the TriKinetics software and investigated hourly from Day 1 (08:00). As

191 the arrays monitored guppy activity every minute, recordings were then averaged per hour, to
192 follow the ZT system.

193 *Experiment 2: Impact of infection on daily activity of guppy shoals*

194 To observe whether there is a difference in activity between uninfected and infected wild-
195 type guppy shoals under a 12:12 h light: dark regime, female adult guppies were size matched
196 (13.21 ± 0.67 mm) into shoals of five individuals ($n = 16$ groups). Each shoal was housed in 6
197 L familiarization tanks for a minimum of 12 days (Griffiths and Magurran, 1997) prior to trials.
198 On Day 1 of the experiment, each familiarised shoal was transferred to a test arena (150 cm
199 length x 20 cm width x 16 cm depth) for a 24 h acclimation period. At 08:00 the following day
200 (Day 2), fish were removed from the arena, and one guppy was anaesthetised and infected
201 with 30 gyrodactylids, whilst the remaining 4 fish in each shoal were sham infected to account
202 for handling time. Fish were placed in individual 1 L pots for 30 min recovery time, whilst
203 remaining in visual contact to one another. On Day 3, an observer (partially hidden by a
204 screen) recorded the proportion of time (sec) an infected and a randomly selected uninfected
205 fish spent actively swimming during a 5-min focal follow over five time points; three diurnal
206 (ZT1: 08:00, ZT6: 13:00 and ZT11: 18:00 h) and two nocturnal (ZT15: 22:00 and ZT18: 01:00
207 h). Fish were deemed actively swimming when propelling themselves forward. After the 5-min
208 focal follow, both fish were screened to account for any parasites transfer. Data collected from
209 the uninfected individuals were used as a control.

210 *Experiment 3: Impact of photoperiod on parasite daily activity*

211 To identify whether there is rhythmical variance in parasite activity under the two light regimes
212 (12:12 h light: dark and 24 h constant darkness; constant darkness often used a 'free-running'
213 condition - a test of endogenous circadian rhythms; Brown et al., 2011), we monitored the
214 host-seeking motion of the parasite (number of probes), which is part of their exploratory
215 behaviour (Bakke et al., 2007). For both light conditions (12:12 h light: dark and 24 h
216 darkness), wild-type juvenile guppies ($n = 60$ for each experiment) were size-matched (10.75
217 ± 0.40 / 11.10 ± 0.9 mm) and each fish infected with two gyrodactylids, before being placed

218 individually in 1 L dechlorinated water pots. After an acclimation period of 7 days, during which
219 parasite number on each host increased naturally, in a 12:12 h light: dark regime, fish were
220 anaesthetised and parasite activity recorded for a 2-min period under a dissecting microscope,
221 using a Longse standard box camera. The activity of three randomly selected parasites on the
222 fins of each fish was analysed. For the first condition, parasite activity was recorded both in
223 light and dark depending on the ZT point, whereas for the second condition at ZT0 the light
224 remained off, so all recordings took place in constant darkness with infrared light. Once
225 recordings concluded, the host parasite load was also recorded. For these observations,
226 timepoints monitored were ZT0 (07:00 h), ZT4 (11:00 h), ZT8 (15:00 h), ZT12 (19:00 h), ZT16
227 (23:00 h) and ZT20 (03:00 h).

228 *Experiment 4: Impact of photoperiod on parasite transmissibility*

229 To examine whether daily variation in parasite activity affected their transmissibility to a new
230 host, ornamental female adult guppies ($n = 120$) were size-matched (12.94 ± 1.3 mm) into
231 dyads. One guppy from each dyad ($n = 60$) was infected with two gyrodactylids and all guppies
232 were placed individually in 1 L pots. After an acclimation period of 7 days in a 12:12 h light:
233 dark regime, infected individuals were screened to determine their parasite load. Then, both
234 infected and uninfected guppies from each dyad were placed together in 25 ml of
235 dechlorinated water for 1 h, resulting in 10 dyads at each of the following time points: ZT0
236 (07:00 h), ZT4 (11:00 h), ZT8 (15:00 h), ZT12 (19:00 h), ZT16 (23:00 h) and ZT20 (03:00 h).
237 After 1 h, fish were separated, anaesthetised and screened to record how many parasites had
238 transferred from the donor to the recipient or how many parasites had been dislodged.

239 *Statistical analysis*

240 All statistical analyses were conducted using R statistical software (version 4.1.1, R Core
241 Team, 2019). To analyse the data, the following packages were used: 'lme4' to run
242 Generalised Linear Mixed Models (GLMMs) (Bates et al., 2015), 'emmeans' for *post hoc*
243 analyses (Searle et al., 1980), 'ggplot2' to visualize data (Wickham, 2009) and 'circacompare'
244 to compare rhythms (Parsons et al., 2020). The 'circacompare' package was used to compare

245 rhythms between different conditions by assessing MESOR, amplitude and acrophase across
246 rhythms. MESOR (Midline Estimating Statistic of Rhythm) refers to the rhythm-adjusted mean
247 level of a response variable around which a wave function oscillates, so the mean activity level
248 over a certain period of time. Amplitude is a measure of half the extent of predictable variation
249 within a cycle, so the activity variation from the MESOR, which is the mean, to the peak of
250 activity. Acrophase refers to the time at which the response variable peaks; time that it takes
251 to go from MESOR to Amplitude (Otsuka et al., 2016; Parsons et al., 2020; Figure 2).

252 For Experiment 1, a GLMM fitted with 'binomial error' family and 'logit' link function
253 assessed activity (count of infrared beam breaks) of infected and uninfected isolated guppies
254 in light and dark conditions. Guppy activity was the dependent term in the model, and fixed
255 effects were infection status (infected or uninfected) and light condition (light or dark). Fish
256 number was included as a random term to account for repeated measures. Additionally, the
257 'circacompare' package was used to investigate and compare the rhythms of infected and
258 uninfected individuals in 12:12 h light: dark regime over a 48 h period. For Experiment 2, one
259 GLMM, fitted with 'binomial error' family and 'logit' link function, was used to assess diel activity
260 patterns of infected and uninfected guppies. In the GLMM, the proportion of time fish remained
261 actively swimming during a 5-minute period was the dependent term in the model, and fixed
262 effects included infection status (infected or uninfected) and ZT as well as an interaction term
263 between infection status and ZT, and the shoal number was included as a random term to
264 account for repeated measures. For Experiment 3, a Generalised Linear Model (GLM) was
265 used to compare parasite activity (number of probes) with respect to different ZT and light
266 conditions. An interaction term between ZT and light conditions was incorporated into the
267 model. Moreover, the 'circacompare' package was used to investigate and compare rhythms
268 of parasite activity in different light conditions. For Experiment 4, two GLMs, fitted with
269 'binomial error' family and 'logit' link function assessed the proportion of parasites transmitting
270 from an infected host to its uninfected conspecific and proportion of parasites that had been
271 dislodged with respect to ZT, light conditions and parasite density on the host. In all tests, the
272 level of significance was taken as $p < 0.05$. GLMM models were refined through stepwise

273 deletion of non-significant terms and AIC comparisons and their robustness was assessed
274 using residual plots, indicating that assumptions of models were met (Pinheiro and Bates,
275 2000). Mean standard length was not included within models, as fish were size-matched at
276 the start of each experiment to eliminate size variability.

277 **Results**

278 Overall, the circadian rhythm detected in guppy activity was disturbed by infection, resulting
279 in increased activity at night, thus nocturnal restlessness both in isolated guppies and in
280 shoals. Even though gyrodactylid behaviour and activity did not exhibit diurnal variance,
281 parasite activity peaked at night, coinciding with the increase in host activity.

282 *Experiment 1: Impact of infection on daily activity of isolated guppies*

283 For both uninfected (control) and infected guppies there was a significant difference in activity
284 between light and dark conditions (emmeans; $df = 1$; $p < 0.0001$ and $p < 0.0001$ respectively;
285 Figure 3a), with both uninfected and infected fish having significantly higher activity in the light
286 comparing to the dark conditions. In light conditions, uninfected guppies were significantly
287 more active than infected guppies (emmeans; $df = 1$; $p = 0.0005$), whilst the opposite was
288 observed in dark conditions, whereby uninfected guppies were less active than their infected
289 conspecifics (emmeans; $df = 1$; $p = 0.036$; Figure 3a). The 'circacompere' package confirmed
290 the presence of circadian rhythmicity in activity of both the uninfected ($p = 0.006$) and infected
291 group ($p = 0.0008$; Figure 3b). The two rhythms had a significant difference in MESOR ($p =$
292 0.0008), with the uninfected group having a greater rhythm-adjusted mean than infected
293 group, in acrophase ($p = 0.0004$) with the uninfected group having an earlier peak and a
294 significantly higher amplitude, which is the half of the predictable variation in activity
295 throughout the 48 h period ($p = 0.0002$; Figure 3b).

296 *Experiment 2: Impact of infection on daily activity in guppy shoals*

297 Swimming activity of uninfected guppies was elevated during the day and dropped drastically
298 at night. When guppies were infected, however, they exhibited nocturnal restlessness with
299 increased swimming activity, indicating that infection status had a significant effect on

300 swimming activity of guppies when in shoals, which also depended on ZT (ZT x Infection status
301 interaction; GLMM; $p < 0.001$). When studying shoal swimming activity at specific ZT
302 timepoints, uninfected guppies were significantly less active than infected conspecifics at each
303 timepoint (GLMM; $df = 4$; $p < 0.001$), evidently more so during nocturnal hours where there is
304 a great difference in activity of uninfected and infected shoaling guppies (ZT15, ZT18; Figure
305 4).

306 *Experiment 3: Impact of photoperiod on parasite daily activity*

307 Light conditions and ZT timepoint both had a significant effect on parasite activity (GLM; $p =$
308 0.007 and $p < 0.001$ respectively) as well as their interaction (Light conditions x ZT timepoints;
309 GLM; $p < 0.001$). Overall, parasites were more active in the dark compared to light conditions
310 under the 12:12 h light: dark regime (GLM; $df = 1$; $p = 0.0004$; Figure 5a). When comparing
311 parasite activity between the 12:12 h light: dark regime and constant darkness (Figure 5b),
312 there was a significant difference in ZT0 (emmeans; $df = 1$; $p < 0.0001$), ZT4 (emmeans; $df =$
313 1 ; $p < 0.0001$), ZT8 (emmeans; $df = 1$; $p = 0.015$), ZT12 (emmeans; $df = 1$; $p = 0.004$), ZT16
314 (emmeans; $df = 1$; $p = 0.009$) but not ZT20 (emmeans; $df = 1$; $p = 0.342$). The 'circacompare'
315 package, however, did not detect a circadian rhythm in parasite activity either in 12:12 h light:
316 dark or 48 h of darkness regime, suggesting that it is not endogenously driven, but affected
317 by other cues (Figure 5b).

318 *Experiment 4: Impact of photoperiod on parasite daily transmissibility*

319 The proportion of parasites that transferred from an infected host to an uninfected
320 conspecific (GLMM; $df = 5$; $p > 0.05$) or the proportion of parasites that dislodged from their
321 host (GLMM; $df = 5$; $p > 0.05$) were not significantly different between ZT timepoints, in light
322 vs. dark conditions or dependent on parasite density of the host. Also, the 'circacompare'
323 package did not detect a rhythm in parasite transmissibility in the 12:12 h light: dark regime (p
324 > 0.05) with no significant difference detected in MESOR, amplitude and phase.

325 **Discussion**

326 Here, we provide the first empirical evidence of aquatic ectoparasites directly altering 'rest-
327 activity' cycles of diurnal fish hosts. Using the guppy-gyrodactylid system, we showed infection
328 changes the daily rhythms of guppy activity; infected individuals were more active at night than
329 their uninfected conspecifics, with nocturnal restlessness exhibited both in isolated and guppy
330 shoals. Although gyrodactylid behaviour (host-searching activity and transmissibility) did not
331 exhibit diurnal cycles, parasites did display elevated activity at night (supporting Brooker et al.,
332 2011). Our results are important because regulated rest-activity cycles are essential for
333 physical and mental wellbeing (Besedovsky et al., 2012) and most notably optimizing efficient
334 immune functioning (Imeri and Opp, 2009). Sleep deprivation can result in cognitive
335 impairment (Alhola and Polo-Kantola, 2007) and increased disease susceptibility (Cohen et
336 al., 2009). Moreover, disease itself induces dramatic sleep alterations, although previously
337 only reported for endoparasite infections (Norman et al., 1990; Toth, 1995; Buguet et al.,
338 1993).

339 Ectoparasites likely inflict some degree of physical discomfort to their hosts during
340 establishment on the host skin and throughout infection. In the case of gyrodactylids, they
341 attach to their host primarily using hooks, and following establishment extrude digestive
342 enzymes onto the hosts' skin from which host epidermal cells and mucus are subsequently
343 ingested (Bakke et al., 2007). The frequent movement of gyrodactylids across the host's skin,
344 potentially associated with their avoidance of localised host immune responses (Richards and
345 Chubb, 1996), may irritate the guppy hosts and result in increased host nocturnal activity. In
346 the case of host activity experiments, both when isolated and in shoals, observations started
347 within 24 h of parasite infection, so shortly after host infection. As also shown in other parasite
348 species, the brain-infecting *Euhaplorchis californiensis* cercariae had an impact on their killifish
349 host (*Fundulus parvipinnis*) during parasite exposure. Host activity and metabolic rate
350 increased, with metabolic rate remaining elevated while activity returned to normal, suggesting
351 ongoing physiological changes are separate from behavioural effects (Nadler et al., 2021). So,

352 migration of gyrodactylids across the host's skin and subsequent irritation may have been a
353 driver of host nocturnal restlessness.

354 Whilst not measured here, complex interactions between immune and hormonal
355 modulation activated by infection may have also contributed to nocturnal restlessness.
356 Inflammatory responses to infection, for example, significantly contribute to sleep disturbances
357 (Ali et al., 2013). In fish, a typical response to ectoparasite infection is epidermal thickening
358 (Esteban et al., 2012; Smallbone et al., 2016), whereby inflammation at the site of parasite
359 establishment occurs after tegument damage (Lindenstrøm et al., 2004). Inflammatory
360 responses are regulated by pro- and anti-inflammatory cytokines, which promote and inhibit
361 rest, respectively. The production and release of pro-inflammatory cytokines has been
362 associated with the secretion of melatonin (Srinivasan et al., 2005): a regulatory hormone
363 essential for enhancing propensity to sleep (Cajochen et al. 2003; Zhdanova et al. 2001).
364 Perturbances in natural oscillations of a protein like melatonin can promote restlessness (Budh
365 et al., 2005). So, disease can induce dramatic rest alterations, so far only emphasized in
366 endoparasite infections (Norman et al., 1990; Toth, 1995; Buguet et al., 1993), leading to a
367 constant state of restlessness. However, previous studies have shown that shortly after
368 infection, effective localised immune responses towards gyrodactylids and infection-related
369 changes in gene expression are exhibited (Bakke et al., 2007; Konczal et al., 2020;
370 Lindenstrøm et al., 2003). Therefore, these disruptions in immune responses could further
371 promote host restlessness. Overall, there is increasing evidence of complex interactions
372 between molecular clocks and immunity (Ellison et al., 2021), as dysregulation of certain host
373 clock proteins linked with cell function, defence and inflammation may lead, among others, to
374 inflammatory diseases and immunodeficiency (Curtis et al., 2014). While clock gene
375 expression drives daily cycles in immunity, immune activation caused by infection can itself
376 alter clock gene expression. Therefore, we suggest the observed changes in daily behaviour
377 patterns could a result of altered clocks.

378 Regarding parasite activity, even though no ‘true’ circadian rhythmicity in phenotypes
379 was detected, host seeking behaviour and activity were greater in the dark and more
380 specifically at the end of the light period (ZT12), where there was a peak in parasite activity.
381 Interestingly, this elevated host seeking behaviour coincides with natural guppy shoaling
382 behaviour peaking around dusk (Croft et al., 2003; O'Connor and Krause, 2003). Thus, an
383 increase in parasite activity could potentially facilitate transmission between hosts, as fish are
384 closely aggregated during shoaling (Pitcher, 1983). Elevated host activity may be beneficial
385 to individuals as infected fish move between and directly contact resting conspecifics
386 (Reynolds et al., 2019), potentially diluting their parasite burdens (Mooring and Hart, 1992).
387 Previous studies demonstrate the benefits of successful parasite transmission in terms of
388 ‘vaccinating’ hosts against subsequent infections (Faria et al., 2010), but also reducing
389 resource competition between parasites and allowing short-term evasion of a hosts’ immune
390 response (Richards and Chubb, 1996), concluding that a driver of parasite activity could be
391 host behaviour.

392 Better understanding of both guppy and gyrodactylid behavioural and activity patterns,
393 provides a greater insight in host-parasite dynamics. Using this knowledge of circadian
394 rhythms may be helpful in tackling infectious diseases, as chronotherapeutic approaches
395 could be used to yield maximum therapeutant efficiency based on host metabolism, when
396 treating for parasites. In aquaculture, parasite infections do not always lead to fish mortality,
397 yet still negatively impact productivity, health and welfare of fish (Shinn et al., 2015), thus
398 extensive use of therapeutics is used to maximise efficiency (Burka et al., 1997; Grant, 2002).
399 However, drug efficacy and toxicity vary with time of day (Bruguerolle, 1998), as daily rhythms
400 in drug absorption, metabolism, detoxification and excretion have been reported in
401 mammalian species (Smolensky and Peppas, 2007). As shown by Vera and Migaud (2016),
402 Atlantic salmon (*Salmo salar*) treated with hydrogen peroxide (H₂O₂) revealed increased
403 sublethal toxic effect during the first half of the day, providing the first evidence of
404 chronotoxicity in Atlantic salmon. Moreover, the impact of photoperiod and infection status
405 on immune gene activation as well as immune expression and rhythmicity was investigated

406 by Ellison et al. (2021), where it was shown that circadian perturbation, that shifts the
407 magnitude and timing of immune activity, is detrimental to fish health. These studies provide
408 evidence for potential optimisation of treatment timing in aquaculture, opening the door to
409 treating fish diseases chronotherapeutically. In addition, non-detected infections, which alter
410 fish behaviour such as increased restlessness, could be used as a diagnostic tool for
411 emerging infectious diseases.

412 In conclusion, we demonstrate that ectoparasites alter daily rhythmic activity of their
413 hosts, resulting in greater nocturnal restlessness both individually and in shoals. Circadian
414 rhythmicity in activity was present and distinctly different between uninfected and infected
415 fish. Peaks in activity may be driven parasite skin irritation as well as immune responses to
416 infection, such as infection resolution and repair, which are elevated at night (Ellison et al.,
417 2021) and may have direct implications for other animal behaviour studies that overlook
418 nocturnal activity of diurnal species. We also provide a better understanding of parasite
419 behaviour, that does not exhibit a daily rhythmical variance, but peaks in the dark, coinciding
420 with infected fish behaviour. As gyrodactylids pose a significant threat to many economically
421 important fish in aquaculture (Lafferty et al., 2015; Shinn et al., 2015), the use and application
422 of chronotherapy to maximize treatment efficacy could be a potential solution to this problem.

423 **Ethical standards**

424 All applicable institutional guidelines for the care and use of animals were followed (Kilkenny
425 et al., 2014). Procedures and protocols were conducted under UK Home Office licence (PPL
426 303424) with approval by the Cardiff University Animal Ethics Committee.

427 **Acknowledgements**

428 We thank Emily Shaw, Benjamin Goodman and Sarah Hendry for technical assistance.

429 **Financial Support**

430 This research was funded by a Knowledge Economy Skills Scholarship II (KESSII) to EA,
431 supported by European Social Funds (ESF) through the Welsh Government. KESS is a pan-
432 Wales higher level skills initiative led by Bangor University on behalf of the HE sector in
433 Wales.

434 **Conflicts of Interest**

435 The authors declare there are no conflicts of interest.

436

437 **References**

- 438 Adam, K. 1980. Sleep as a restorative process and a theory to explain why. In: *Progress in*
439 *Brain Research*. Elsevier, pp. 289–305.
- 440 Adams, K.L., Castanon-Cervantes, O., Evans, J.A. and Davidson, A.J., 2013. Environmental
441 circadian disruption elevates the IL-6 response to lipopolysaccharide in blood. *Journal of*
442 *Biological Rhythms*, 28(4), pp.272-277.
- 443 Alhola, P. and Polo-Kantola, P. 2007. Sleep deprivation: Impact on cognitive
444 performance. *Neuropsychiatric Disease and Treatment* 3(5), pp. 553–567.
- 445 Ali, T., Choe, J., Awab, A., Wagener, T.L. and Orr, W.C. 2013. Sleep, immunity and
446 inflammation in gastrointestinal disorders. *World journal of gastroenterology: WJG*, 19(48),
447 p.9231.
- 448 Bakke, T.A., Cable, J. and Harris, P.D. 2007. The Biology of Gyrodactylid Monogeneans: The
449 “Russian-Doll Killers”. In: *Advances in Parasitology*. Elsevier, pp. 161–460.
- 450 Bass, J. and Lazar, M.A. 2016. Circadian time signatures of fitness and
451 disease. *Science* 354(6315), pp. 994–999.
- 452 Bates, D, Mächler, M, Bolker, B and Walker, S. 2015. Fitting linear mixed-effects models
453 using lme4. *Journal of Statistical Software* 67, 1–48.
- 454 Benington, J.H. and Craig Heller, H. 1995. Restoration of brain energy metabolism as the
455 function of sleep. *Progress in Neurobiology* 45(4), pp. 347–360.
- 456 Besedovsky, L., Lange, T. and Born, J. 2012. Sleep and immune function. *Pflügers Archiv-*
457 *European Journal of Physiology*, 463(1), pp.121-137.
- 458 Boeuf, G. and Le Bail, P.Y. 1999. Does light have an influence on fish
459 growth? *Aquaculture* 177(1–4), pp. 129–152.
- 460 Brooker, A.J., Grano Maldonado, M.I., Irving, S., Bron, J.E., Longshaw, M. and Shinn, A.P.
461 2011. The effect of octopaminergic compounds on the behaviour and transmission of
462 *Gyrodactylus*. *Parasites & Vectors* 4(1), p. 207.
- 463 Brown, M.A., Quan, S.F. and Eichling, P.S. 2011. Circadian rhythm sleep disorder, free-
464 running type in a sighted male with severe depression, anxiety, and agoraphobia. *Journal of*
465 *clinical sleep medicine: JCSM: official publication of the American Academy of Sleep*
466 *Medicine* 7(1), pp. 93–94.

- 467 Bruguerolle, B. 1998. Chronopharmacokinetics: Current Status. *Clinical*
468 *Pharmacokinetics* 35(2), pp. 83–94.
- 469 Bryant, P.A., Trinder, J. and Curtis, N. 2004. Sick and tired: does sleep have a vital role in the
470 immune system? *Nature Reviews Immunology* 4(6), pp. 457–467.
- 471 Budh, C.N., Hultling, C. and Lundeberg, T. 2005. Quality of sleep in individuals with spinal
472 cord injury: a comparison between patients with and without pain. *Spinal Cord* 43(2), pp. 85–
473 95.
- 474 Buguet, A., Bert, J., Tapie, P., Tabaraud, F., Doua, F., Lonsdorfer, J., Bogui, P. and Dumas,
475 M. 1993. Sleep-wake cycle in human African trypanosomiasis. *Journal of clinical*
476 *neurophysiology: official publication of the American Electroencephalographic Society*, 10(2),
477 pp.190-196.
- 478 Burka, J.F., Hammell, K.L., Horsberg, T.E., Johnson, G.R., Rainnie, D.J. and Speare, D.J.
479 1997. Drugs in salmonid aquaculture – A review. *Journal of Veterinary Pharmacology and*
480 *Therapeutics* 20(5), pp. 333–349.
- 481 Cable, J. and van Oosterhout, C. 2007. The impact of parasites on the life history evolution of
482 guppies (*Poecilia reticulata*): The effects of host size on parasite virulence. *International*
483 *Journal for Parasitology* 37(13), pp. 1449–1458.
- 484 Cable, J., Scott, E.C.G., Tinsley, R.C. and Harris, P.D. 2002. Behavior favoring transmission
485 in the viviparous monogenean *Gyrodactylus turnbulli*. *Journal of Parasitology* 88(1), pp. 183–
486 184.
- 487 Cajochen, C., Kräuchi, K. and Wirz-Justice, A. 2003. Role of melatonin in the regulation of
488 human circadian rhythms and sleep: melatonin, sleep and circadian rhythms. *Journal of*
489 *Neuroendocrinology* 15(4), pp. 432–437.
- 490 Castanon-Cervantes, O., Wu, M., Ehlen, J.C., Paul, K., Gamble, K.L., Johnson, R.L., Besing,
491 R.C., Menaker, M., Gewirtz, A.T. and Davidson, A.J., 2010. Dysregulation of inflammatory
492 responses by chronic circadian disruption. *The Journal of Immunology*, 185(10), pp.5796-
493 5805.
- 494 Chambel, J., Pinho, R., Sousa, R., Ferreira, T., Baptista, T., Severiano, V., Mendes, S. and
495 Pedrosa, R. 2015. The efficacy of MS-222 as anaesthetic agent in four freshwater aquarium
496 fish species. *Aquaculture Research*, 46(7), pp.1582-1589.
- 497 Cirelli, C. and Tononi, G. 2008. Is sleep essential? *PLoS Biology* 6(8), p. e216.

- 498 Cohen, S., Doyle, W.J., Alper, C.M., Janicki-Deverts, D. and Turner, R.B. 2009. Sleep habits
499 and susceptibility to the common cold. *Archives of Internal Medicine* 169(1), p. 62.
- 500 Croft, D.P., Arrowsmith, B.J., Bielby, J., Skinner, K., White, E., Couzin, I.D., Magurran, A.E.,
501 Ramnarine, I. and Krause, J. 2003. Mechanisms underlying shoal composition in the
502 Trinidadian guppy, *Poecilia reticulata*. *Oikos*, 100(3), pp.429-438.
- 503 Curtis, A.M., Bellet, M.M., Sassone-Corsi, P. and O'Neill, L.A.J. 2014. Circadian clock proteins
504 and immunity. *Immunity* 40(2), pp. 178–186.
- 505 Davidson, A.J. 2006. Search for the feeding-entrainable circadian oscillator: a complex
506 proposition. *American Journal of Physiology-Regulatory, Integrative and Comparative*
507 *Physiology* 290(6), pp. R1524–R1526.
- 508 Doube, B.M. 1975. Cattle and the paralysis tick *Ixodes Holocyclus*. *Australian Veterinary*
509 *Journal* 51(11), pp. 511–515.
- 510 DuRant, S.E., Hopkins, W.A., Davis, A.K. and Romero, L.M. 2015. Evidence of ectoparasite-
511 induced endocrine disruption in an imperilled giant salamander, the eastern hellbender
512 (*Cryptobranchus alleganiensis*). *Journal of Experimental Biology*, p. jeb.118703.
- 513 Ellison, A.R., Wilcockson, D. and Cable, J. 2021. *Circadian dynamics of the teleost skin*
514 *immune-microbiome interface*. *Microbiology*.
- 515 Ernst, I. and Whittington, I.D. 1996. Hatching rhythms in the capsalid monogeneans
516 *Benedenia lutjani* from the skin and *B. rohdei* from the gills of *Lutjanus carponotatus* at Heron
517 Island, Queensland, Australia. *International Journal for Parasitology* 26(11), pp. 1191–1204.
- 518 Esteban, M., Castaño, A., Schindler, B., Koch, H., Angerer, J., Casteleyn, L., Joas, R., Joas,
519 A., Biot, P., Aerts, D. and Kolossa-Gehring, M. 2012. S-150: Strategies and Tools to Obtain
520 Comparable Biomarker Results in the Pan-European COPHES Human Biomonitoring
521 Project. *Epidemiology*, 23(5S).
- 522 Faria, P.J., van Oosterhout, C. and Cable, J. 2010. Optimal release strategies for captive-bred
523 animals in reintroduction programs: Experimental infections using the guppy as a model
524 organism. *Biological Conservation* 143(1), pp. 35–41.
- 525 Gannicott, A.M. and Tinsley, R.C. 1997. Egg hatching in the monogenean gill parasite
526 *Discocotyle sagittata* from the rainbow trout (*Oncorhynchus mykiss*). *Cambridge University*
527 *Press* 114(6), pp. 569–579.
- 528 Grant, A.N. 2002. Medicines for sea lice. *Pest Management Science* 58(6), pp. 521–527.

529 Harris, P.D., Shinn, A.P., Cable, J. and Bakke, T.A. 2004. Nominal species of the genus
530 *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host
531 species. *Systematic Parasitology* 59(1), pp. 1–27.

532 Helvig, A., Wade, S. and Hunter-Eades, L. 2016. Rest and the associated benefits in
533 restorative sleep: a concept analysis. *Journal of Advanced Nursing* 72(1), pp. 62–72.

534 Ibarra-Coronado, E.G., Pantaleón-Martínez, A.M., Velazquez-Moctezuma, J., Prospéro-
535 García, O., Méndez-Díaz, M., Pérez-Tapia, M., Pavón, L. and Morales-Montor, J., 2015. The
536 bidirectional relationship between sleep and immunity against infections. *Journal of*
537 *immunology research*, pp. 1–14.

538 Imeri, L. and Opp, M.R. 2009. How (and why) the immune system makes us sleep. *Nature*
539 *Reviews Neuroscience* 10(3), pp. 199–210.

540 Kearns, G.C. 1967. Experiments on host-finding and host-specificity in the monogenean skin
541 parasite *Entobdella soleae*. *Parasitology* 57(3), pp. 585–605.

542 Kilkenny, C., Browne, W., Cuthill, I., Emerson, M. and Altman, D. 2014. Improving bioscience
543 research reporting: the ARRIVE guidelines for reporting animal research. *Animals* 4(1), pp.
544 35–44.

545 Kolluru, G.R., Grether, G.F., Dunlop, E. and South, S.H. 2009. Food availability and parasite
546 infection influence mating tactics in guppies (*Poecilia reticulata*). *Behavioural Ecology* 20(1),
547 pp. 131–137.

548 Konczal, M., Ellison, A.R., Phillips, K.P., Radwan, J., Mohammed, R.S., Cable, J. and
549 Chadzinska, M. 2020. RNA-Seq analysis of the guppy immune response against *Gyrodactylus*
550 *bullatarudis* infection. *Parasite Immunology* 42(12).

551 Kripke, D.F., Garfinkel, L., Wingard, D.L., Klauber, M.R. and Marler, M.R. 2002. Mortality
552 associated with sleep duration and insomnia. *Archives of General Psychiatry* 59(2), p. 131.

553 Krueger, J.M., Frank, M.G., Wisor, J.P. and Roy, S. 2016. Sleep function: Toward elucidating
554 an enigma. *Sleep Medicine Reviews* 28, pp. 46–54.

555 Lafferty, K.D., Harvell, C.D., Conrad, J.M., Friedman, C.S., Kent, M.L., Kuris, A.M., Powell,
556 E.N., Rondeau, D. and Saksida, S.M. 2015. Infectious diseases affect marine fisheries and
557 aquaculture economics. *Annual review of marine science*, 7, pp.471-496.

558 Leung, L.C., Wang, G.X., Madelaine, R., Skariah, G., Kawakami, K., Deisseroth, K., Urban,
559 A.E. and Mourrain, P. 2019. Neural signatures of sleep in zebrafish. *Nature*, 571(7764),
560 pp.198-204.

561 Liang, X., Bushman, F.D. and FitzGerald, G.A. 2015. Rhythmicity of the intestinal microbiota
562 is regulated by gender and the host circadian clock. *Proceedings of the National Academy of*
563 *Sciences* 112(33), pp. 10479–10484.

564 Lindenstrøm, T., Buchmann, K. and Secombes, C.J. 2003. *Gyrodactylus derjavini* infection
565 elicits IL-1 β expression in rainbow trout skin. *Fish & Shellfish Immunology* 15(2), pp. 107–115.

566 Lindenstrøm, T., Secombes, C.J. and Buchmann, K. 2004. Expression of immune response
567 genes in rainbow trout skin induced by *Gyrodactylus derjavini* infections. *Veterinary*
568 *immunology and immunopathology*, 97(3-4), pp.137-148.

569 Majde, J. and Krueger, J. 2005. Links between the innate immune system and sleep. *Journal*
570 *of Allergy and Clinical Immunology* 116(6), pp. 1188–1198.

571 Midttun, H.L., Vindas, M.A., Whatmore, P.J., Øverli, Ø. and Johansen, I.B., 2020. Effects of
572 *Pseudoloma neurophilia* infection on the brain transcriptome in zebrafish (*Danio rerio*). *Journal*
573 *of fish diseases*, 43(8), pp.863-875.

574 Mideo, N., Reece, S.E., Smith, A.L. and Metcalf, C.J.E. 2013. The Cinderella syndrome: why
575 do malaria-infected cells burst at midnight? *Trends in Parasitology* 29(1), pp. 10–16.

576 Mooring, M.S. and Hart, B.L. 1992. Animal grouping for protection from parasites: selfish herd
577 and encounter-dilution effects. *Behaviour* 123(3–4), pp. 173–193.

578 Nadler, L.E., Bengston, E., Eliason, E.J., Hassibi, C., Helland-Riise, S.H., Johansen, I.B.,
579 Kwan, G.T., Tresguerres, M., Turner, A.V., Weinersmith, K.L. and Øverli, Ø., 2021. A brain-
580 infecting parasite impacts host metabolism both during exposure and after infection is
581 established. *Functional Ecology*, 35(1), pp.105-116.

582 Norman, S.E., Chediak, A.D., Kiel, M. and Cohn, M.A. 1990. Sleep disturbances in HIV-
583 infected homosexual men: *AIDS* 4(8), pp. 775–782.

584 O'Connor, E. and Krause, J. 2003. Effect of light intensity on the shoaling behaviour of the
585 guppy (*Poecilia reticulata*). *Journal of Fish Biology* 63, pp. 254–254.

586 O'Donnell, A.J., Schneider, P., McWatters, H.G. and Reece, S.E. 2011. Fitness costs of
587 disrupting circadian rhythms in malaria parasites. *Proceedings of the Royal Society B:*
588 *Biological Sciences* 278(1717), pp. 2429–2436.

589 Opp, M.R. 2009. Sleep and psychoneuroimmunology. *Immunology and Allergy Clinics of*
590 *North America* 29(2), pp. 295–307.

591 Otsuka, K., Cornelissen, G., Furukawa, S., Kubo, Y., Hayashi, M., Shibata, K., Mizuno, K.,
592 Aiba, T., Ohshima, H. and Mukai, C. 2016. Long-term exposure to space's microgravity alters
593 the time structure of heart rate variability of astronauts. *Heliyon*, 2(12), p.e00211.

594 Parsons, R., Parsons, R., Garner, N., Oster, H. and Rawashdeh, O. 2020. CircaCompare: a
595 method to estimate and statistically support differences in mesor, amplitude and phase,
596 between circadian rhythms. Valencia, A. ed. *Bioinformatics*, p. btz730.

597 Penev, P.D., Kolker, D.E., Zee, P.C. and Turek, F.W. 1998. Chronic circadian
598 desynchronization decreases the survival of animals with cardiomyopathic heart
599 disease. *American Journal of Physiology-Heart and Circulatory Physiology* 275(6), pp.
600 H2334–H2337.

601 Piggins, H.D. 2002. Human clock genes. *Annals of Medicine* 34(5), pp. 394–400.

602 Pinheiro, J.C. and Bates, D.M. 2000. Extending the basic linear mixed-effects model.
603 In: *Mixed-Effects Models in S and S-PLUS*. Statistics and Computing. New York: Springer-
604 Verlag, pp. 201–270.

605 Pitcher, T. J. 1983. Heuristic definitions of shoaling behaviour. *Animal Behaviour*. 31, 611-
606 163.

607 Preston, B.T., Capellini, I., McNamara, P., Barton, R.A. and Nunn, C.L. 2009. Parasite
608 resistance and the adaptive significance of sleep. *BMC Evolutionary Biology* 9(1), p. 7.

609 R Core Team. 2019. R: A Language and Environment for Statistical Computing. Vienna,
610 Austria: *R Foundation for Statistical Computing*.

611 Reeb, S.G. 2002. Plasticity of diel and circadian activity rhythms in fishes. *Reviews in Fish*
612 *Biology and Fisheries* 12(4), pp. 349–371.

613 Reynolds, M., Hockley, F.A., Wilson, C.A.M.E. and Cable, J. 2019. Assessing the effects of
614 water flow rate on parasite transmission amongst a social host. *Hydrobiologia* 830(1), pp.
615 201–212.

616 Richards, G.R. and Chubb, J.C. 1996. Host response to initial and challenge infections,
617 following treatment, of *Gyrodactylus bullatarudis* and *G. turnbulli* (Monogenea) on the guppy
618 (*Poecilia reticulata*). *Parasitology Research* 82(3), pp. 242–247.

619 Rijo-Ferreira, F., Pinto-Neves, D., Barbosa-Morais, N.L., Takahashi, J.S. and Figueiredo, L.M.
620 2017. *Trypanosoma brucei* metabolism is under circadian control. *Nature Microbiology* 2(6),
621 p. 17032.

622 Rinaldi, L., Russo, T., Schioppi, M., Pennacchio, S. and Cringoli, G. 2007. *Passalurus*
623 *ambiguus*: new insights into copromicroscopic diagnosis and circadian rhythm of egg
624 excretion. *Parasitology Research* 101(3), pp. 557–561.

625 Schelkle, B., Shinn, A., Peeler, E. and Cable, J. 2009. Treatment of gyrodactylid infections in
626 fish. *Diseases of Aquatic Organisms* 86, pp. 65–75.

627 Searle, S.R., Speed, F.M. and Milliken, G.A. 1980. Population marginal means in the linear
628 model: an alternative to least squares means. *The American Statistician* 34(4), pp. 216–221.

629 Shinn, A.P., Pratoomyot, J., Bron, J., Paladini, G., Brooker, E.E. and Brooker, A.J. 2015.
630 Economic impacts of aquatic parasites on global finfish production. *Global Aquaculture*
631 *Advocate*, pp.58-61.

632 Siegel, J.M. 2008. Do all animals sleep? *Trends in Neurosciences* 31(4), pp. 208–213.

633 Smallbone, W., van Oosterhout, C. and Cable, J. 2016. The effects of inbreeding on disease
634 susceptibility: *Gyrodactylus turnbulli* infection of guppies, *Poecilia reticulata*. *Experimental*
635 *Parasitology* 167, pp. 32–37.

636 Smolensky, M.H. and Peppas, N.A. 2007. Chronobiology, drug delivery, and
637 chronotherapeutics. *Advanced Drug Delivery Reviews* 59 (9–10), pp. 828–851.

638 Sollars, P.J. and Pickard, G.E. 2015. The neurobiology of circadian rhythms. *Psychiatric*
639 *Clinics of North America* 38(4), pp. 645–665.

640 Srinivasan, V., Maestroni, G.J., Cardinali, D.P., Esquifino, A.I., Perumal, S.R. and Miller, S.C.
641 2005. Melatonin, immune function and aging. *Immunity & Ageing*, 2(1), pp.1-10.

642 Stewart, A., Jackson, J., Barber, I., Eizaguirre, C., Paterson, R., van West, P., Williams, C.
643 and Cable, J. 2017. Hook, line and infection: a guide to culturing parasites, establishing
644 infections and assessing immune responses in the three-spined stickleback. *Advances in*
645 *parasitology*, 98, pp.39-109.

646 Takahashi, K., Lin, J.S. and Sakai, K. 2008. Neuronal activity of orexin and non-orexin waking-
647 active neurons during wake–sleep states in the mouse. *Neuroscience* 153(3), pp. 860–870.

648 Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.A.,
649 Dufour, S., Karlsten, Ø., Norberg, B. and Andersson, E. 2010. Control of puberty in farmed
650 fish. *General and comparative endocrinology*, 165(3), pp.483-515.

651 Theron, A. and Combes, C. 1995. Asynchrony of infection timing, habitat preference, and
652 sympatric speciation of schistosome parasites. *Evolution* 49(2), p. 372.

653 Toth, L.A. 1995. Sleep, sleep deprivation and infectious disease: Studies in
654 animals. *Advances in Neuroimmunology* 5(1), pp. 79–92.

655 Van Oosterhout, C., Harris, P.D. and Cable, J. 2003. Marked variation in parasite resistance
656 between two wild populations of the Trinidadian guppy, *Poecilia reticulata* (Pisces:
657 Poeciliidae): Parasite Resistance in The Trinidadian Guppy. *Biological Journal of the Linnean*
658 *Society* 79(4), pp. 645–651.

659 Vera, L.M. and Migaud, H. 2016. Hydrogen peroxide treatment in Atlantic salmon induces
660 stress and detoxification response in a daily manner. *Chronobiology International* 33(5), pp.
661 530–542.

662 Whiting, J.R., Mahmud, M.A., Bradley, J.E. and MacColl, A.D.C. 2020. Prior exposure to long-
663 day photoperiods alters immune responses and increases susceptibility to parasitic infection
664 in stickleback. *Proceedings of the Royal Society B: Biological Sciences* 287(1930), p.
665 20201017.

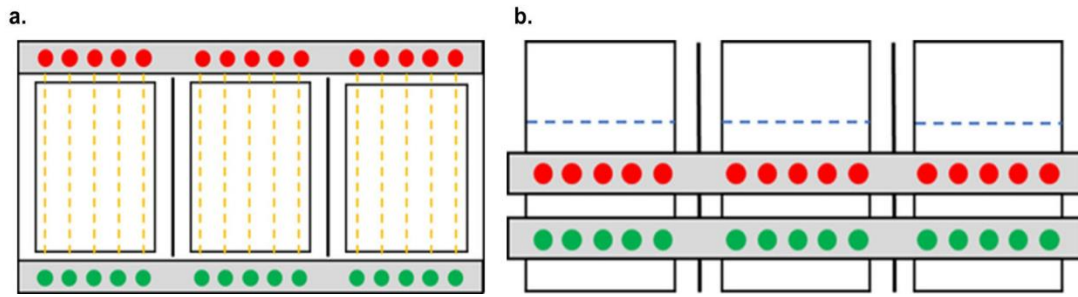
666 Widder, E.A., Robison, B.H., Reisenbichler, K.R. and Haddock, S.H.D. 2005. Using red light
667 for in situ observations of deep-sea fishes. *Deep Sea Research Part I: Oceanographic*
668 *Research Papers* 52(11), pp. 2077–2085.

669 Zhdanova, I.V., Wang, S.Y., Leclair, O.U. and Danilova, N.P. 2001. Melatonin promotes sleep-
670 like state in zebrafish. *Brain Research* 903(1–2), pp. 263–268.

671 Zietara, M.S. and Lumme, J. 2002. Speciation by host switch and adaptive radiation in a fish
672 parasite genus *Gyrodactylus* (monogenea, Gyrodactylidae). *Evolution* 56(12), pp. 2445–
673 2458.

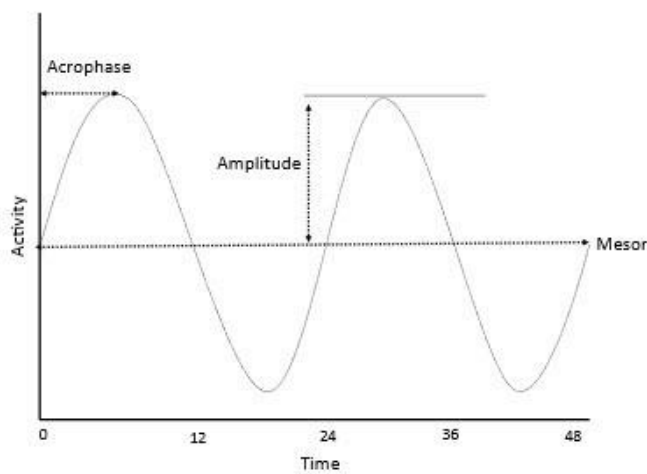
674

675
676
677
678
679
680
681



682 **Figure 1.** 2D schematic showing the set-up of the behavioural arrays for Experiment 1. **a.** Birds eye
683 view of the behavioural arrays with 2 rows of five infrared beams (yellow dotted line) going through each
684 fish tank from the light emitters (green) to the light receivers (red). **b.** Side view of the behavioural arrays
685 with two rows of monitors outside of each tank with the light emitters going through the tank to the
686 receivers on the other side, with light emitters and receivers alternating between rows. The water level
687 is indicated (blue dotted line) along with the paper dividers between the tanks (black line).

688
689
690
691
692
693
694
695
696



697 **Figure 2.** Variables assessed by the 'circacompare' package in each rhythm and then compared
698 between rhythms (include Mesor, the rhythm-adjusted mean level; amplitude, half the extend of
699 predictable variation; and acrophase the time the response variable peaks).

700
701
702
703

704
 705
 706
 707
 708
 709
 710
 711
 712
 713
 714
 715
 716
 717
 718
 719
 720
 721
 722
 723
 724
 725
 726
 727
 728
 729
 730
 731
 732
 733
 734

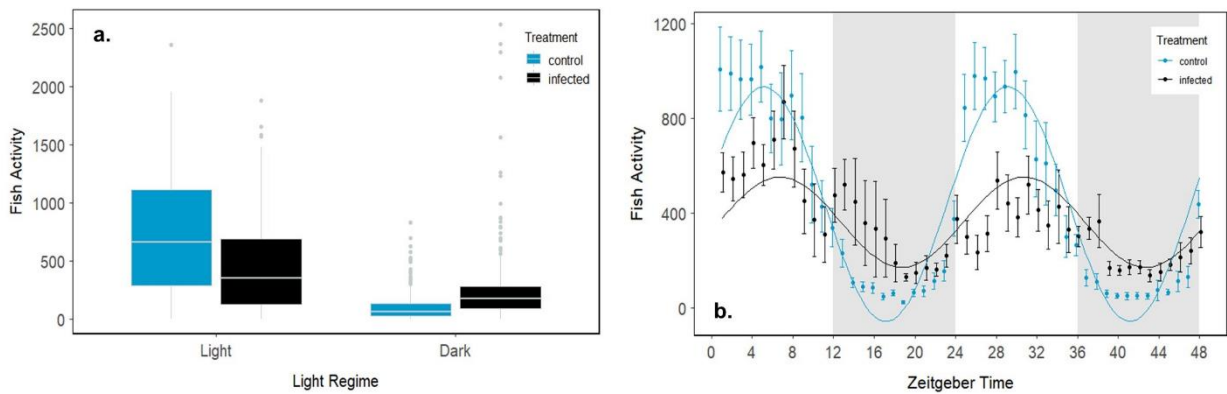


Figure 3.a. Activity of isolated guppies from uninfected and *Gyrodactylus turnbulli* infected fish in light and dark conditions. In the light, the uninfected guppies were significantly more active than the infected fish and in the dark uninfected guppies were significantly less active than their infected conspecifics. Fish activity is defined as the number of times fish went through the infrared beams per tank, as retrieved from the TriKinetics software. Dots represent outliers; the box the first and third quartile with median and the line 50% of fish activity. **b.** The activity of uninfected and infected guppies monitored hourly for a 47 h period. Grey areas indicate dark periods. Error bars represent standard error.

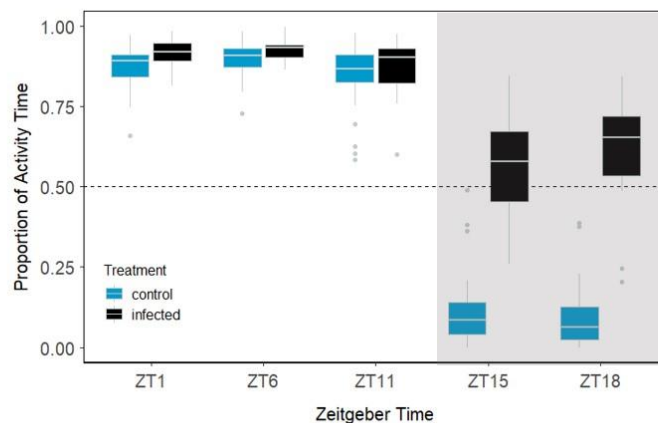


Figure 4. The proportion of time *Gyrodactylus turnbulli* infected and uninfected guppies remained active during 5-min focal follows at five ZT timepoints. Grey areas indicate dark periods. Black dots represent outliers; bars the upper and lower limits; the box the first and third quartile with median, and the dashed line 50% of the time in which guppies remained active during a focal follow.

735

736

737

738

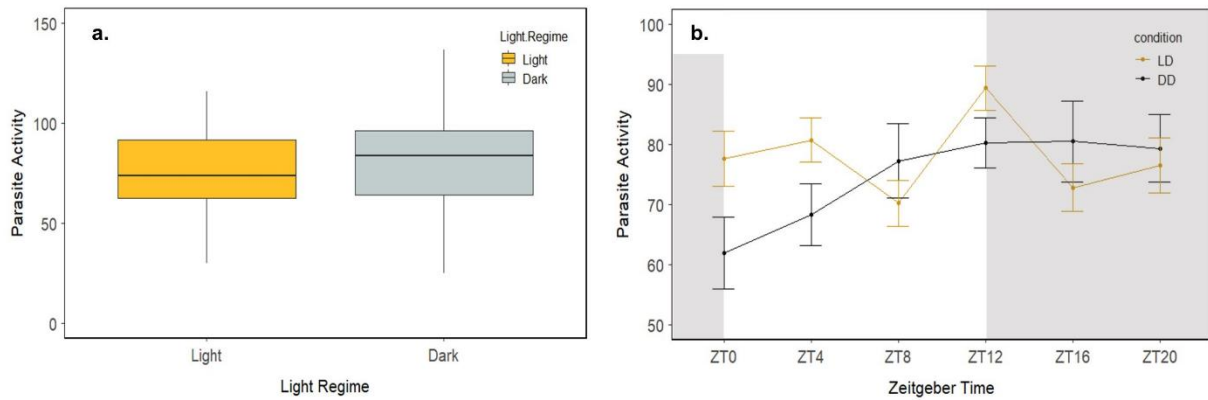
739

740

741

742

743



744 **Figure 5.a.** Activity (number of probes) of *Gyrodactylus turnbulli* parasites present on their guppy host

745 in light and dark conditions. The box represents the first and third quartile with median. **b.** Parasite

746 activity recorded both in 12:12 h light: dark regime (LD) and 48 h constant darkness (DD). There was

747 significant difference in activity at ZT0, ZT4, ZT8, ZT12 and ZT16. However, there was no rhythmicity

748 detected in either case. Grey areas indicate dark periods. Error bars represent standard error.

749

750

751

752

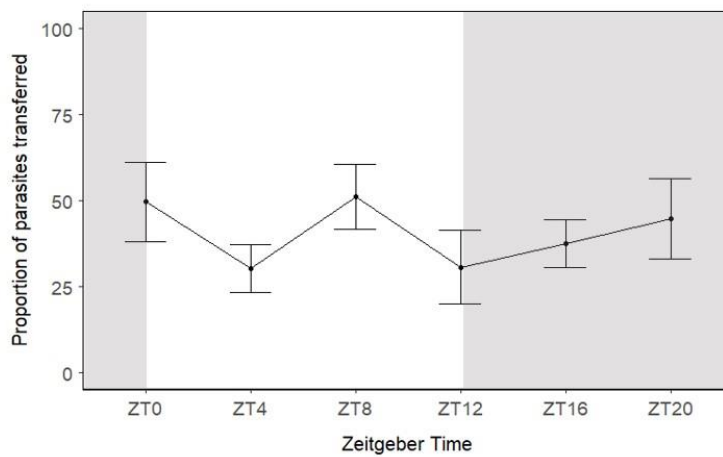
753

754

755

756

757



758

759

760 **Figure 6.** Proportion of parasites that transferred from the host to the recipient conspecific at different

761 ZT points in a 12:12 h light: dark regime with no significant difference recorded. Grey areas indicate

762 dark periods. Error bars represent standard error.