Shining a light on parasite behaviour: daily patterns of Argulus fish lice

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1   Shining a light on parasite behaviour: daily patterns of *Argulus* fish lice

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8

9   **Abstract**

10 Parasites display a wide range of behaviours that are frequently overlooked in favour of host

11 responses. Understanding these behaviours can improve parasite control through more precise

12 application or development of new behaviour-based strategies. In aquaculture fish lice are an

13 ongoing problem, infections reduce fishery production and control options are limited. Fish

14 lice are distinct in their ability to survive and swim off hosts, allowing transmission to multiple

15 fish hosts across their lifespan. Here we assessed off host behaviour of *Argulus foliaceus* (a

16 freshwater fish louse) and observed a diurnal rhythmical pattern in behaviour. This pattern was

17 lost when lice were exposed to constant darkness, indicating that the behaviour is not

18 endogenously driven. Males were consistently active in light with reduced activity in darkness.

19 In contrast, females were active during light and dark phases with peak activity at the start of

20 dark periods. *A. foliaceus* was also strongly attracted to a light stimulus, preferring white and

21 blue coloured light to green or red. Light is a strong driver of fish louse activity and could be

22 used to trap parasites. Aquaculture light regimes could also be altered to reduce parasite

23 attraction and activity.

24

25 **Key words:** Parasite Behaviour, Fish lice, Circadian, Aquaculture

26

Key Findings

28 • Fish lice have daily rhythmical behaviour that differs between males and females.

29 • This behaviour is triggered by light changes and is not endogenously driven.

30 • Fish lice are strongly attracted to shorter wavelengths of light.

31

Introduction

Parasites are a fundamental component of ecosystems; practically all known species carry parasites and food webs can be dominated by their presence (Marcogliese and Cone 1997; Poulin and Morand 2000; Lafferty *et al.* 2006; Dobson *et al.* 2008). In addition to their ecological importance, parasitic infections play a critical role in the global health of humans and both domesticated and wild species. Conflict between humans and parasites drives development and use of control strategies to prevent and reduce the health and socio-economic impacts of infection. Understanding behaviour can aid development and employment of control strategies, but research tends to focus on host rather than parasite behaviours (Barnard and Behnke 1990; Sukhdeo and Chappell 1994; Lewis *et al.* 2002). This is despite that fact that parasites have developed a wide range of complex behaviours to facilitate transmission, infection, reproduction, and survival (Rea and Irwin 1994; Sukhdeo and Chappell 1994; Lewis *et al.* 2002). Behaviours involved in host finding are of particular interest regarding development of control strategies to interrupt and prevent infection. Many parasites have adopted 'active' host finding behaviours to locate suitable hosts, whereby a parasite responds to environmental and/or host signals (Rea and Irwin 1994). Parasites utilise a range of stimuli (such as chemical, thermal, mechanical and visual), often in combination to locate hosts and assess their suitability (Van Leerdam *et al.* 1985; Ashton *et al.* 1999; Bailey *et al.* 2006; Mordue (Luntz) and Birkett 2009).

Organisms can temporally synchronise to their environment by detecting and responding

52 to external cues, resulting in biological rhythms of physiology and behaviour (Vitaterna *et al.*

53 2001; Bell-Pedersen *et al.* 2005). Light-dark cycles are the dominant cue for a majority of

54 organisms, however for parasites both environmental and host cues influence rhythmicity

55 (Bell-Pedersen *et al.* 2005; Reece *et al.* 2017). By synchronising with hosts, parasites can

56 increase their survival. During dispersal and transmission, rhythms allow parasites to maximise

57 infection success by optimizing presence of infective stages with host availability (Sukhdeo

58 and Chappell 1994; Bogéa *et al.* 1996). In addition, infection success and parasite survival can

59 be influenced by fluctuations (daily and/or seasonal) in host immune responses (Martinez-

60 Bakker and Helm 2015; Kiessling *et al.* 2017; Carvalho Cabral *et al.* 2019). Identification of

61 cues used by parasites and the rhythms they exhibit could help reduce infection and

62 transmission risks; for example, by avoiding/preventing access to locations during peak

63 parasite presence or deploying control measures at such times to maximise capture.

64   The environmental and/or host cues utilised by parasites can differ between life stage or

65 sexes. While this can reduce the efficacy of broad control applications and induce bias, it can

66 also be used for highly targeted control. Sex specific control schemes have been employed to

67 successfully reduce parasite populations or their vectors. Females can be targeted to reduce the

68 next generation by directing removing reproducers, while male targeting uses sterilisation and

69 release techniques to lower population fecundity (Alphey *et al.* 2010; Epsky *et al.* 1999).

70 Discrete sexes can be caught using sex-specific behaviours such as pheromone or food-based

71 attraction (Epsky *et al.* 1999). These sex-specific behaviours likely lead to sex specific

72 rhythms, which could also be exploited to further promote control success. Sexual differences

73 in parasite rhythms have yet to be explored (Sikkel *et al.* 2009).

74   Fish lice are ectoparasitic crustaceans, problematic worldwide in fisheries. Control options

75 are limited, with reduction in chemical applications due to environmental concerns and rising

76 drug resistance (Taylor *et al.* 2005a; Costello 2009). Recent developments in control of marine

77   sea lice capitalises on louse behaviour: lice frequent the top of the water column, consequently

78   fish are held at >10 m below sea surface to reduce infection. For freshwater lice (genus *Argulus*)

79   however, control options remain insufficient with some farmers turning to illegal options

80   (Taylor *et al.* 2005b). Thus, there is a need to explore alternative, behaviour-based control

81   methods. *Argulus* spp. are unusual in that they retain the ability to free swim throughout their

82   life cycle with host switching frequent, especially among male parasites as they seek female

83   partners (Bandilla *et al.* 2008). No studies have tested for the presence of endogenous rhythms

84   in *Argulus* spp. (or any other aquatic ectoparasitic crustacean), although a diurnal pattern is

85   present in the strength of their positive phototaxis response (Yoshizawa and Nogami 2008).

86   *Argulus* spp. also react to light/dark changes with differing activity, however this has not been

87   observed over a circadian period or between sexes (Mikheev *et al.* 1999).

88   Here we examine host seeking behaviour of a globally problematic fish ectoparasite over

89   a diurnal period, testing for the presence of endogenous cues. Strength of light attraction and

90   wavelength specific preferences are also assessed to aid control development.

91

92   **Materials and methods**

93   *Parasite and host maintenance*

94   *Argulus* spp. used in this study were collected from Risca Canal (Newport, UK; grid reference:

95   ST 24344 90686) on 06/06/18 and 07/08/19 by hand netting naturally infected three-spined

96   stickleback *Gasterosteus aculeatus*. Parasites were removed from fish in the field by lifting the

97   host fish out of water using a net for a 10 s period; upon re-submersion into a container of

98   freshwater the parasite detached and was collected using a wide-bore pipette. *Argulus* spp. were

99   transported to the lab off host in sealed containers of dechlorinated water. Once in the Cardiff

100 aquarium, parasites were morphologically identified as *A. foliaceus* (according to Fryer 1982)

101 and maintained in male: female pairs on three-spined sticklebacks collected from Roath Brook,

102   Cardiff (ST 18897 78541; an *Argulus* spp. naïve population). Fish were infected by placing the

103   parasites into fish holding tanks (9 L) containing an individual stickleback to allow natural

104   parasite attachment. All fish and parasites were maintained under a 12 h light:12 h dark cycle,

105   with fish fed daily with *Tubifex* bloodworm. *A. foliaceus* were acclimated to laboratory

106   conditions on their hosts for 1 week prior to experimentation. Parasites were not re-used within

107   or across experiments. For the circadian rhythm experiment, both male and non-gravid female

108   parasites were used. For the light attraction/colour preference experiments only male *A.*

109   *foliaceus* were used due to higher availability of male parasites versus non-gravid females (as

110   female parasites continuously produce eggs after mating and egg baring females exhibit egg

111   laying behaviour when off host).

112   *A. foliaceus* were removed from sticklebacks for use in experiments using the same

113   collection method as described above. All *A. foliaceus* were checked visually for damage

114   before use and measured from the rostral edge of the carapace to the anterior end of the

115   abdominal lobes using a dissecting microscope at 10x magnification with a Lumenera Infinity

116   1 camera and Infinity Capture software version 6.5.4.

117   The experimental procedures in this study conform to the accepted principles of animal

118   welfare in experimental science and used the minimum number of animals required to produce

119   statistically reproducible results. All animal work was approved by the Cardiff University's

120   Animal Ethics Committee, followed ARRIVE guidelines and was conducted under Home

121   Office License PPL 303424.

122

123   *Circadian Rhythm of parasite swimming activity off host*

124   To understand how *A. foliaceus* behave off host/during transmission over a circadian period,

125   individual adult male and non-gravid female *A. foliaceus* (males: N = 22, average size = 3.93

126   mm ± 0.23 SD, females: N = 18, average size = 4.43 mm ± 0.44 SD,) were placed into glass

127   petri dishes (10 cm diameter) filled with 50 ml dechlorinated water. The water level in the petri

128   dishes was sufficient to allow full horizontal movement, while minimizing vertical motion for

129   behavioural tracking. Additionally, the sides of each dish were covered with white fabric to

130   reduce reflections and prevent visual disturbance (Mikheev *et al.* 1998). Parasites were then

131   subject to 12 h light:12 h dark (LD; average 1000 lux) for 48h, after which they were removed

132   from the setup, given one day of recovery on stickleback hosts (to allow feeding/prevent

133   starvation) before returning to the setup for another 48h under total darkness (DD). The order

134   of light condition (12:12 LD vs DD) could not be randomised as the total darkness regime

135   would disrupt any entrained circadian rhythm, altering any tests post exposure. The setup was

136   completely reset between trials and light condition tests. Parasite behaviour was recorded

137   during the 48 h exposures via 24 h infrared CCTV cameras (Sentient Pro HDA DVR 8 Channel

138   CCTV, Maplin). Every 4 h (zeitgeber time = ZT, ZT0 = 7 am, ZT4 = 11 am, ZT8 = 3 pm, ZT12

139   = 7 pm, ZT16 = 11 pm, ZT20 = 3 am; lights on at ZT0 and off at ZT12) the total distance

140   covered by the parasite and subsequent average swimming speed was calculated over a 2 min

141   period using ImageJ version 1.51j8 (Schneider *et al.* 2012) to prepare video files for analysis

142   and Kinovea version 0.8.27 (Ganni *et al.* 2018) to track parasite movement. Proportion of time

143   spent swimming was obtained from Kinovea by calculating the time spent swimming at >1

144   mm/s (approximately ¼ body length). Patterns of parasite activity were then assessed for a 24

145   h period and between 12:12 LD/DD trials to determine activity and entrainment of rhythm.

146

147   *Argulus light attraction in the presence of fish hosts*

148   The attraction of *A. foliaceus* to a light source versus a live fish host was assessed using two

149   different behavioural assays: fish vs light trials in which adult male *A. foliaceus* were given the

150   choice of either a white light or a stickleback in darkness over a 24 h period (N = 20 parasites,

151   average size = 4.12 mm ± 0.31 SD; Fig. 1A), and lit fish vs dark fish trials offering the choice

152   of a stickleback with a white light or a stickleback in darkness (N = 18 parasites, average size

153   = 4.14 mm ± 0.35 SD; Fig. 1B) over a 2 h period. Arenas comprised of glass tank filled to 10

154   cm water depth, split into three identical sized sections (left, middle and right) using a 1 cm

155   aperture mesh to allow free movement of parasites while restricting fish movement (Fig. 1A

156   and B). Stimuli were placed into the left and right thirds, with two *A. foliaceus* restrained under

157   a glass dish in the middle third for 30 min to allow acclimation. After acclimation, the lice were

158   released and monitored via infrared CCTV cameras. All light stimuli used a waterproof LED

159   white light (average 50 lux at a distance of 7 cm), while all stimuli in darkness contained the

160   same type of LED white light but turned off to ensure each section had the same structure. The

161   positions of the stimuli were swapped in between trials to avoid any potential side bias. For the

162   lit fish vs dark fish trials, all host pairs were sized matched.

163

164   *Argulus light colour preference*

165   To investigate whether certain wavelengths of light are more attractive to *A. foliaceus,* adult

166   males (N = 20, average size = 4.08 mm ± 0.33 SD) were placed individually into the centre of

167   a 2.5 L opaque white square arena (14 x 14 cm) filled with 1 L water (5 cm water depth). The

168   arena was split into four equal quarters, with four waterproof lights (3 x 3 x 2 cm, LED with

169   RGB colour) placed into the arena and positioned flush inside each corner (Fig. 1C). Lights

170   were randomly assigned to emit either red (635–700 nm), green (520–560 nm), blue (450–490

171   nm), or white (emits all wavelengths, 450-700 nm) light, with brightness controlled so each

172   light individually generated an average 50 lux (lux meter positioned 7 cm away from light).

173   There was no visual overlap in the colours emitted from each light, and initial testing found

174   that parasites did not swim erratically or behave in any other abnormal manner in the

175   experimental arena (following previous observations in the lab and by Mikheev *et al.* 1998).

176   The inclusion of an acclimation period in initial testing also had no impact on parasite

behaviour, thus parasites were observed immediately after introduction to the arena. After being introduced to the centre of the arena, parasites were monitored for 2 min with their time at each colour recorded. Location at a colour was classified as the parasite being present anywhere in the quarter containing the light (with more than half of the parasites body present in the quarter for when the parasite crossed between sections). Parasites were observed live, with the observer stationed next to the arena looking down into the tank. Room lights were turned off so the only light source during experimentation came from the lights in the arena - this provided enough light to observe parasite movement while preventing casting of shadows into the arena from the observer. Individual parasites were tested 3 times consecutively with average time spent in each light corner calculated. Parasites experienced a rest period of a few seconds between replicates as the arena was reset and light position randomised for each replicate. Parasites did not linger or remain stationary on boundary lines between quarters during observations.

Statistical Analysis

All statistical analyses were conducted using R statistical software (v3.6.2; R Core Team 2017) with the level of significance in all tests taken as $p < 0.05$. Models were refined through stepwise deletion of insignificant terms and AIC comparisons, with visual examination of model plots to check standardised residuals for normal distribution and homogeneity of variance (Crawley 2007). The following packages were used for analyses: "ggplot2" to visualise data (Wickham 2009), "lme4" to run general linear mixed models (Bates *et al.* 2014), "emmeans" for post-hoc analyses (Searle *et al.* 1980), "RAIN" and "MetaCycle" to determine circadian rhythmicity (Thaben and Westermark 2014; Wu *et al.* 2016) and "circacompare" to compare rhythms (Parsons *et al.* 2020). For all rhythm analysis the time period being examined was set to 24 h.

202   To detect rhythmicity, RAIN was used due to its capability in detecting and accounting for

203   asymmetrical patterns (Thaben and Westermark 2014) alongside MetaCycle due to its

204   inclusion of multiple methods for rhythm evaluation (Wu *et al.* 2016). The test "rainresult" was

205   used to examine patterns across parasite sex and light condition by examining phase and peak

206   shape. The phase of a rhythm refers to the time point at which a peak occurs, with peak shape

207   the time (in this case hours) between a peak and the next trough. Comparison of rhythms

208   between different conditions were then investigated using circacompare to assess MESOR

209   (Midline Estimating Statistic of Rhythm), amplitude and phase across rhythms. MESOR is a

210   mean value adjusted for circadian rhythms, amplitude refers to "a measure of half the extent of

211   predictable variation within a cycle" (Cornelissen 2014; Otsuka *et al.* 2016). A general linear

212   mixed model (GLMM) using only the 12:12 LD data was then conducted to compare activity

213   at each ZT time point by examining *A. foliaceus* activity against ZT time, parasite sex and

214   length with an interaction between ZT time/parasite sex. This GLMM was then repeated using

215   the DD trials only. All GLMMs used parasite ID as a random factor to account for

216   pseudoreplication. To determine *A. foliaceus* colour preference, a general linear model was

217   used to compare swimming activity (average over 3 trials) against light colour and parasite

218   length. Across all tests and trials, parasite length had no significant impact and is thus not

219   reported further.

220

221   **Results**

222   *Circadian Rhythm of parasite swimming activity off host*

223   A strong diurnal pattern in off host swimming activity was observed for both male and female

224   *Argulus foliaceus* when maintained under 12:12 LD conditions (RAIN $P =$ <0.001 for both

225   males and females, MetaCycle $P =$ <0.001/0.004 for males/females respectively; Fig. 2);

226   however under total darkness (DD) this diurnal rhythm was lost (RAIN $P = 0.529/0.202$,

227 MetaCycle P = 0.894/0.999 for males/females respectively), suggesting this pattern is

228 stimulated by light and not endogenously driven. Under 12:12 LD, male parasites had different

229 phase to females (circacompare P = 0.018, male phase = 5.69 h post ZT0, female = 8.56 h), but

230 there was no difference in MESOR or amplitude (circacompare P = 0.290/0.716 respectively;

231 Fig. 3).

232   Under 12:12 LD, overall average swimming speed of *A. foliaceus* did not differ among

233 sexes (0.77 and 0.83 cm/s for males and females respectively, GLMM P = 0.591), however

234 when directly comparing ZT timepoints females had a significantly higher swim speed at ZT12

235 (7pm when the lights turn off; GLMM P = 0.008; Fig. 2). Under DD, females had marginally

236 significant higher overall activity than males (0.86 cm/s for females, 0.62 cm/s for males;

237 GLMM P = 0.049). When examining the proportion of time spent swimming, no patterns were

238 observed except for females under DD which showed a peak at ZT0/20 and drop at ZT8/12

239 (females under DD: Rain P = 0.005, MetaCycle P = 0.037, all other treatments: RAIN P =

240 >0.456, MetaCycle P = >0.956; Supplementary Fig. 1).

241

242 *Argulus light attraction in the presence of fish hosts*

243 When assessing preference between a light stimulus or a fish host, the average time taken for

244 lice to first enter the light section was 59 s. After 24 h, 85% of parasites were located at the

245 light stimulus and the remaining 15% had been consumed by the fish host (time to consumption

246 ranged from 11 s - 378 s). No fish became infected during these trials.

247 For trials assessing preference between a fish host with or without a light source turned on,

248 100% of parasites moved to the section containing a fish host with a light on. After 2 h, 17%

249 of these parasites had been eaten by the fish, 22% infected the fish and 61% remained

250 swimming around this section.

251

252 *Argulus light colour preference*

253 *A. foliaceus* significantly preferred white and blue coloured light over green or red (all

254 comparisons $P = <0.001$, except white vs green in which $P = 0.025$), with preference for blue

255 light over white close to significance ($P = 0.052$; Fig.4).

256

257 **Discussion**

258 During dispersal, hosts provide a spatially patchy environment in which parasites need to

259 anticipate host availability (Skelton *et al.* 2015). As such, parasites must develop strategies to

260 increase host-parasite contact and facilitate infection and transmission. In many parasites this

261 involves host-seeking behaviours and synchronisation with their hosts. For fish lice, hosts are

262 located by free-swimming parasites responding to host and environmental cues, with light their

263 dominant stimulus (Bandilla *et al.* 2007). While previous studies have recorded variations in

264 fish lice behaviour over diurnal periods (Yoshizawa and Nogami 2008; Heuch *et al.* 2011),

265 none have determined if these rhythms are endogenously driven. Here *Argulus foliaceus* off

266 host activity followed a diurnal, not endogenous, circadian pattern as the distinct behavioural

267 rhythm under light/dark conditions was lost under total darkness. There was also a sexual

268 difference in off host behaviour with male and female rhythms offset by approximately 4 h.

269 When examining light attraction *A. foliaceus* consistently displayed a strong attraction to light

270 over combined host cues (in the form of a live host) and preferred shorter wavelengths of light.

271   *Argulus* display sexually dimorphic host switching behaviour with males frequently

272 leaving their hosts to find mates while non-gravid females remain on host (Bandilla *et al.* 2008).

273 This dimorphism continues in off host behaviour. As shown previously by (Mikheev *et al.*

274 1999), female *A. foliaceus* had highest activity when the lights turned off and low activity when

275 lights turned on. Examining activity over a circadian period however indicates that this is not

276 sustained, 4+ hours after lights turn off female parasite activity drops, and inversely 4+ hours

277   after lights turn on female activity increases. Males do not follow the same pattern with activity

278   consistently higher during light periods and lower during dark periods. The continued high

279   average speed of females when lights turn off (versus a drop in activity for males) could be

280   related to their host switching behaviours: females are not predisposed to spending time off

281   host, and thus may not react as quickly as males to light changes. Alternatively, the lights used

282   in this study (and Mikheev *et al.* 1999) turned on/off immediately and could be simulating a

283   passing shadow (a trigger of fish lice activity, Bohn 1910; Poulin *et al.* 1990). Females could

284   react stronger than males to potential host cues (due to a higher tendency for females to remain

285   on the host) resulting in high activity when lights turn off. The distinct and strong diurnal

286   rhythm observed when using average swimming speed measurements was not observed when

287   using measurements that only record time spent active. Average swim speed is more

288   comprehensive accounting for variation in activity, while time spent active (i.e. a simple

289   proportion of time moving or not) cannot discern these nuances and would lead to assumption

290   of arrhythmic behaviour. This highlights the importance of selecting the correct activity

291   measure when assessing rhythmical patterns in behaviour.

292   Light is an integral component of aquaculture systems, with differing light wavelengths,

293   intensity and photo periods used to manipulate fish growth and maturation (Boeuf and Le Bail

294   1999; Oppedal *et al.* 1999; Villamizar *et al.* 2011). The subsequent impact of these altered light

295   regimes on both fish behaviour and health is now being considered. Recent studies have also

296   found parasitic infection can alter host circadian gene expression, further complicating the

297   relationship between parasites, hosts and the rhythms they both follow (Ellison *et al.* 2018,

298   2020). Considering the positive phototactic response of fish lice, aquaculture lights could

299   attract lice to cages and facilitate infection (Trippel 2010, Stewart *et al.* 2013). In this study

300   male *A. foliaceus* were more active under light versus dark, suggesting lit cages would not only

301   attract lice but also increase their activity which could lead to higher infection success. Shifting

302   the wavelength of light used in aquaculture systems could potentially allow retention of fish

303   manipulation, while limiting the impact on pathogenic organisms. For example when inhibiting

304   *Salmo salar* sexual maturation to increase production, green and red light treatments used less

305   energy versus white light treatments (Leclercq *et al.* 2011). Additionally, *Oncorhynchus mykiss*

306   raised under red light showed improved growth compared to fish raised under blue or white

307   light (Karakatsouli *et al.* 2008). Red light was the least attractive light colour to *A. foliaceus*

308   (and *A. japonicus*: Yoshizawa and Nogami 2008), therefore cages lit with red light could attract

309   less parasites to those lit with shorter wavelengths. This may only be beneficial in outdoor

310   systems where wild parasites enter containers/cages to infect fish, versus enclosed systems

311   where parasites may be trapped in with the fish.

312   In addition to altering the light regimes in aquaculture to reduce parasite attraction and

313   infection, light could be used to purposefully attract parasites into traps. Light traps have

314   successfully captured sea lice in both the lab and field (where, in comparison, plankton tows

315   captured none) and were suggested as a monitoring tool (Novales Flamarique *et al.* 2009).

316   Unlike sea lice which show differing reaction strength to light across their life stages, *Argulus*

317   spp. appear to be consistent in their light attraction from hatching to adulthood (Bai 1981;

318   Novales Flamarique *et al.* 2000, 2009; Bandilla *et al.* 2007). Additionally, freshwater habitats

319   used for aquaculture are often smaller, enclosed areas (e.g. rearing ponds and raceways,

320   recreational fishing lakes and reservoirs) compared to the ocean, potentially increasing the

321   chance of *Argulus* spp. to encounter traps. Therefore, light traps could be more effective and a

322   feasible management tool for freshwater fisheries and aquaculture. Our findings suggest that

323   over relatively short distances lice are strongly attracted to light, therefore future studies should

324   examine the attraction distance of light coupled with trials in freshwater aquaculture systems

325   to determine the efficacy of light traps in controlling lice infections.

Parasite behaviour can be complex and diverse with host cues, external stimulus and diurnal rhythms all affecting parasite activity. When developing control strategies, understanding behaviour allows more effective application (i.e. during parasite emergence) and offers the potential for identifying new targets for control. Sexual differences are also critical to consider, as differing behaviour could lead to one sex avoiding control application. By understanding and manipulating parasites the impact of infection on global health and economics can be reduced. Parasite behaviour is therefore an important component of management and should be considered for all problematic infections.

Supplementary material
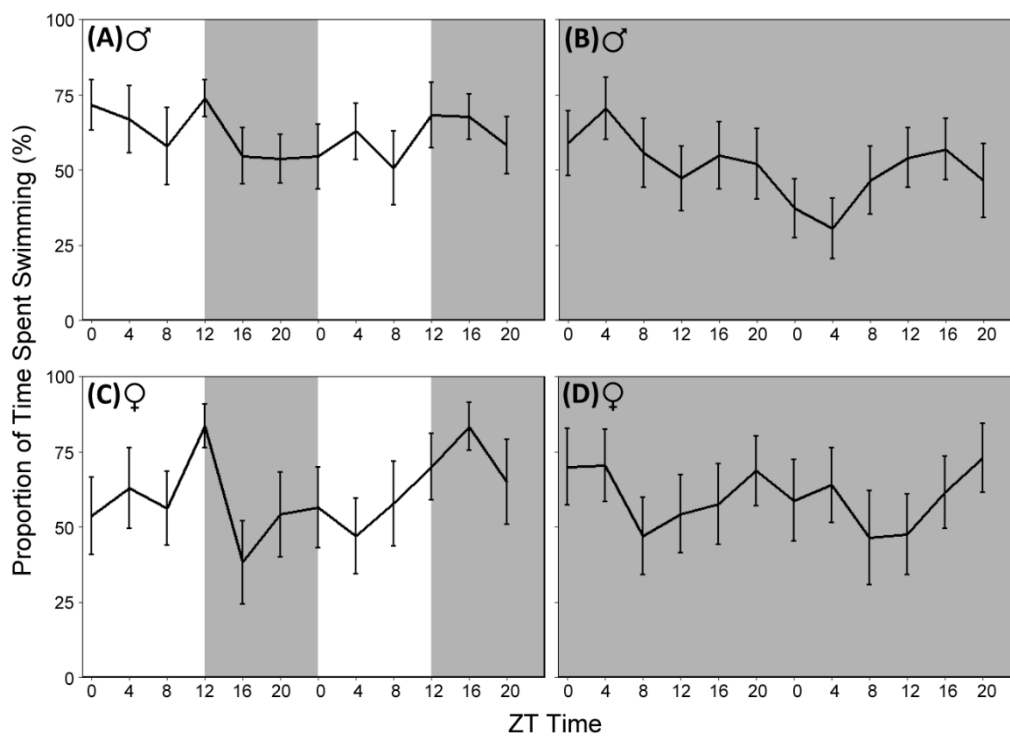
Supplementary figure 1. Proportion of time *Argulus foliaceus* spent swimming off host over a 48h period under differing light conditions. (A) Male *A. foliaceus* under 12h light:12h dark. (B) Male *A. foliaceus* under total darkness. (C) Female *A. foliaceus* under 12h light:12h dark. (D) Female *A. foliaceus* under total darkness. White backgrounds indicate periods of light, dark grey backgrounds indicate periods of darkness. ZT0 = 7am, ZT12 = 7pm.

342

Data

Data will be made available upon request.

345

Acknowledgements

348

Author Contribution

RH, JC and AE conceived and designed the study and co-wrote the article. RH gathered all data and performed the statistical analyses.

352

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359

Conflicts of Interest

The authors declare there are no conflicts of interest.

362

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory

366　animals. All animal work was approved by the Cardiff University's Animal Ethics Committee,

367　followed ARRIVE guidelines and was conducted under Home Office License PPL 303424.

368

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542

Figures

544
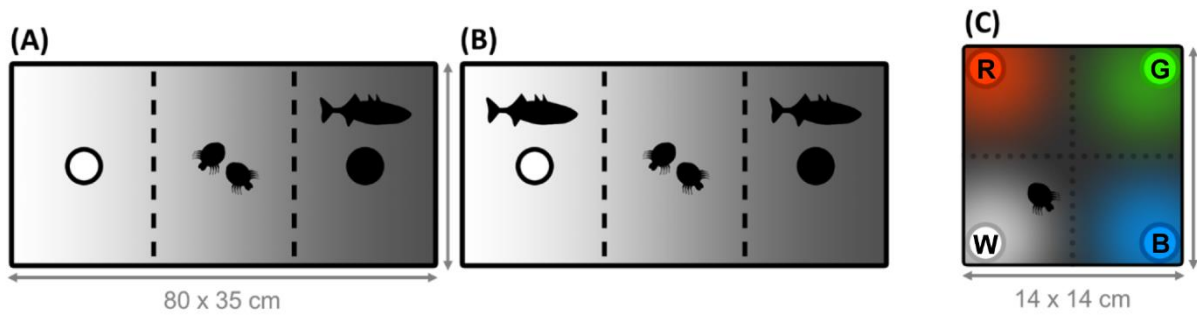
Figure 1. Plan view of experimental areas for *Argulus foliaceus* (A/B) light vs fish host

preference and (C) light colour preference trials. In each arena circles represent LED light

sources. (A) gives a choice of white light vs a three-spined stickleback (*Gasterosteus*

aculeatus) host with a turned off light, (B) gives a choice of a white light + stickleback vs a

turned off light + stickleback. In (A/B) dashed lines represent 1cm aperture mesh which allows

the parasites to swim through while blocking fish movement. In (C) dotted lines indicate the

total area of each coloured corner for behavioural recording, R = red light, G = green, W =

white and B = blue (coloured light placement was changed/randomised for each trial).

553
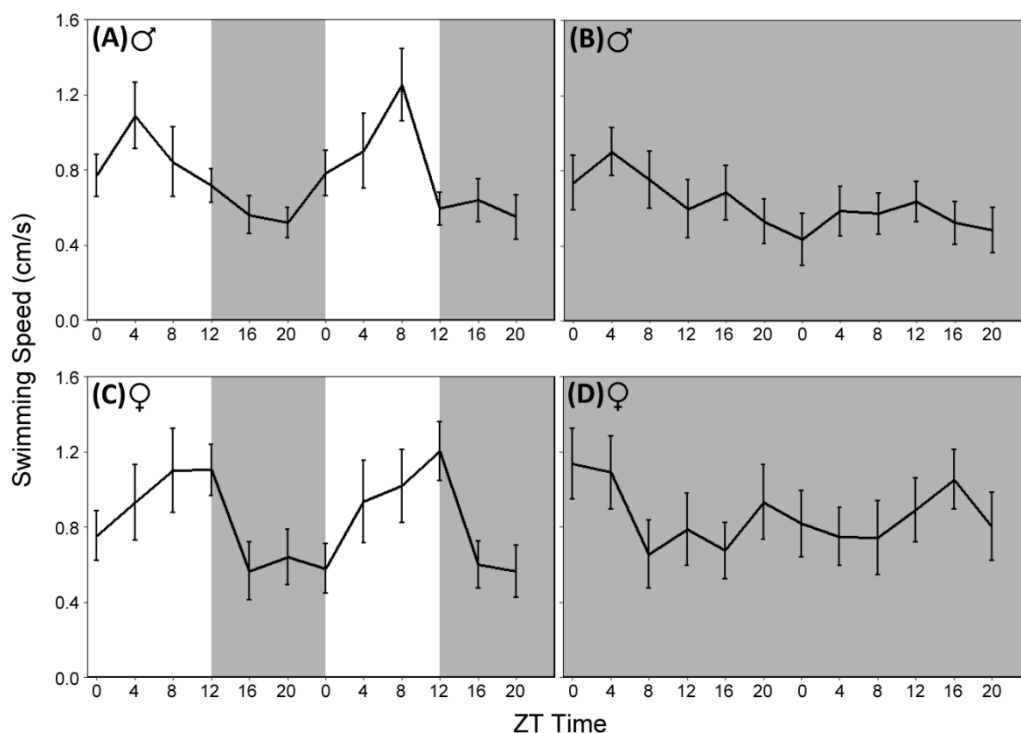
554

555 **Figure 2.** Average swimming speed of *Argulus foliaceus* off host over a 48 h period under two

556 different light conditions: alternating light and dark (A and C) and total darkness (B and D).

557 (A) Male *A. foliaceus* under 12h light:12h dark. (B) Male *A. foliaceus* under total darkness. (C)

558 Female *A. foliaceus* under 12h light:12h dark. (D) Female *A. foliaceus* under total darkness.

559 White backgrounds indicate periods of light, dark grey backgrounds indicate periods of
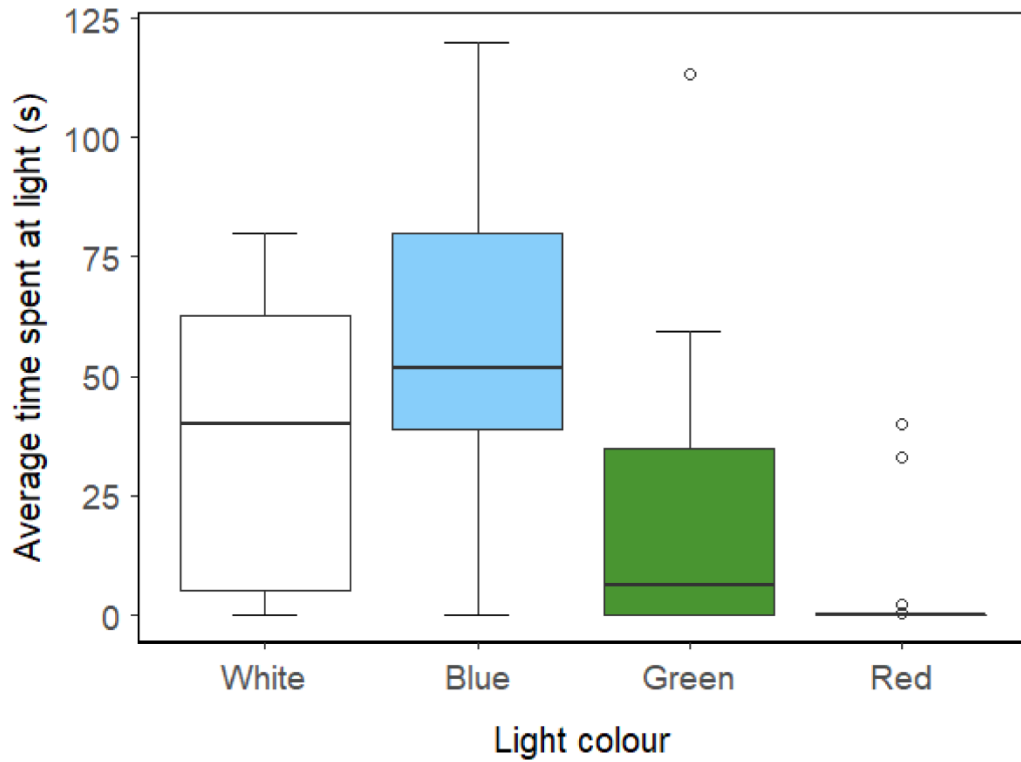
560 darkness. ZT0 = 7am, ZT12 = 7pm.

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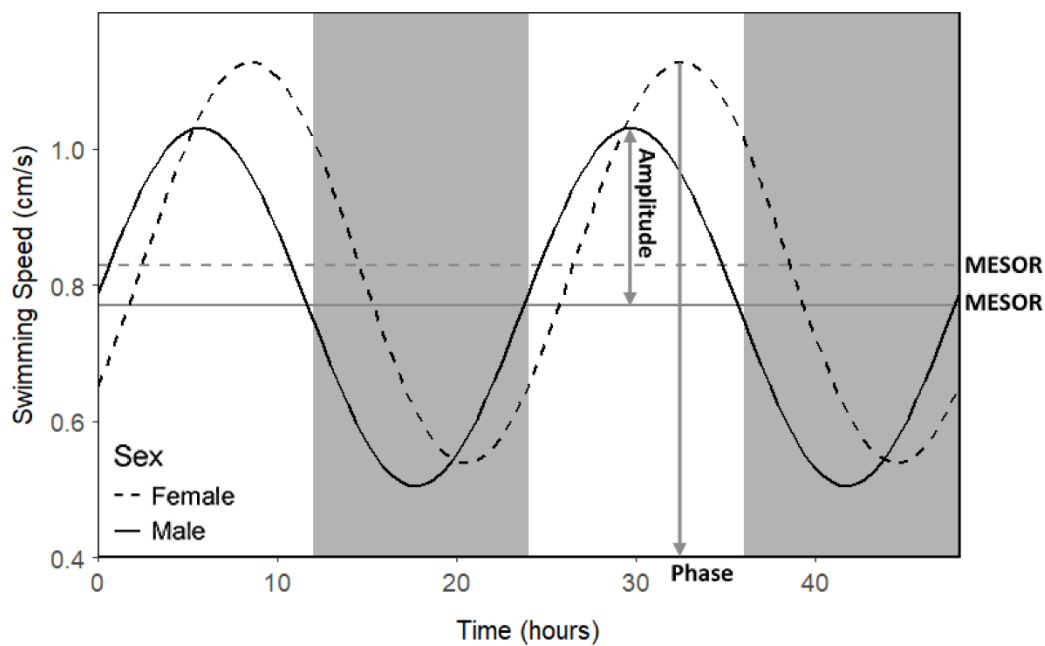
562

563 **Figure 3.** Circacompare output plot of male and female *Argulus foliaceus* swimming speed

564 over a 12:12 light:dark 48h period. Lights turn on/off at 0/12 and 24/36.

565

Figure 4. Light preference of male *Argulus foliaceus* (n = 20) off the host. Average time spent by free-swimming *A. foliaceus* in the vicinity of different coloured lights over a 2-minute period. White light wavelength = 450-700 nm, blue = 450-490 nm, green = 520-560 nm, red = 635-700 nm.