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

2 Rhi Hunt^{1*}, Jo Cable¹ and Amy Ellison²

3

4 ¹ School of Biosciences, Cardiff University, Cardiff, CF10 3AX, U.K. and ² School of Natural
5 Sciences, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, U.K.

6

7 * **Author for correspondence:** Rhi Hunt, E-mail: HuntRC1@cardiff.ac.uk

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

27 **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

32 **Introduction**

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

51 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 bearing 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 \pm 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 \pm 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

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

190

191 *Statistical Analysis*

192 All statistical analyses were conducted using R statistical software (v3.6.2; R Core Team 2017)
193 with the level of significance in all tests taken as $p < 0.05$. Models were refined through
194 stepwise deletion of insignificant terms and AIC comparisons, with visual examination of
195 model plots to check standardised residuals for normal distribution and homogeneity of
196 variance (Crawley 2007). The following packages were used for analyses: “ggplot2” to
197 visualise data (Wickham 2009), “lme4” to run general linear mixed models (Bates *et al.* 2014),
198 “emmeans” for post-hoc analyses (Searle *et al.* 1980), “RAIN” and “MetaCycle” to determine
199 circadian rhythmicity (Thaben and Westermark 2014; Wu *et al.* 2016) and “circacompare” to
200 compare rhythms (Parsons *et al.* 2020). For all rhythm analysis the time period being examined
201 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

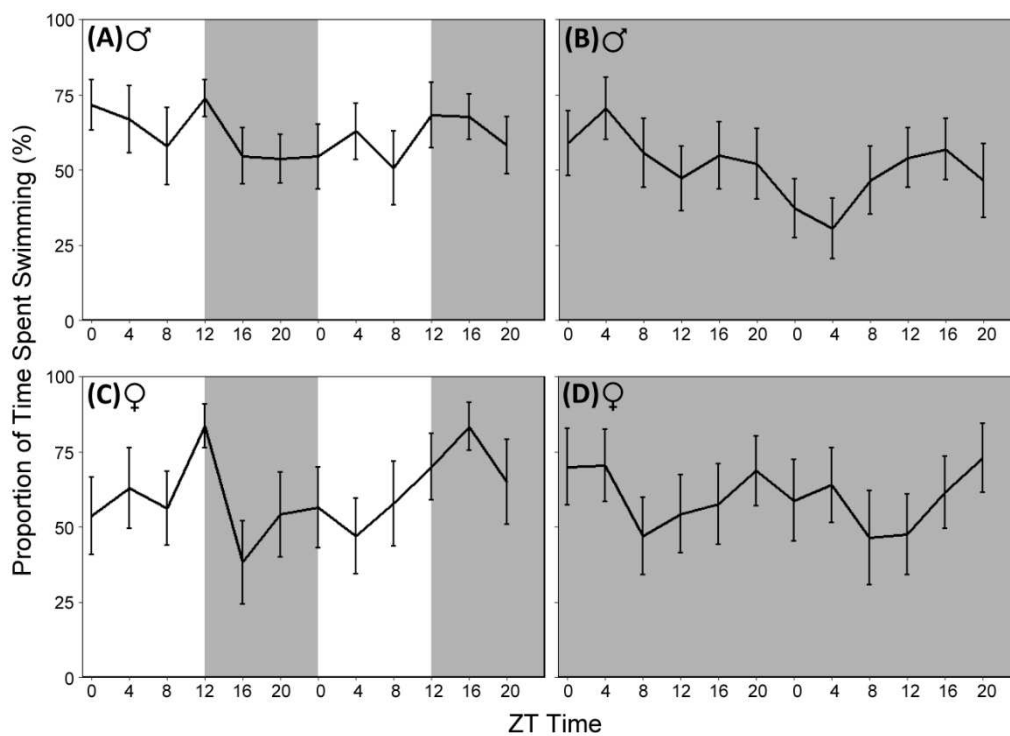
302 the wavelength of light used in aquaculture systems could potentially allow retention of fish
303 manipulation, while limiting the impact on pathogenic organisms. For example when inhibiting
304 *Salmo salar* sexual maturation to increase production, green and red light treatments used less
305 energy 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.

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

334

335 **Supplementary material**



336

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

342

343 **Data**

344 Data will be made available upon request.

345

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348

349 **Author Contribution**

350 RH, JC and AE conceived and designed the study and co-wrote the article. RH gathered all

351 data and performed the statistical analyses.

352

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358 interpretation and analysis, manuscript preparation or decision to publish.

359

360 **Conflicts of Interest**

361 The authors declare there are no conflicts of interest.

362

363 **Ethical Standards**

364 The authors assert that all procedures contributing to this work comply with the ethical

365 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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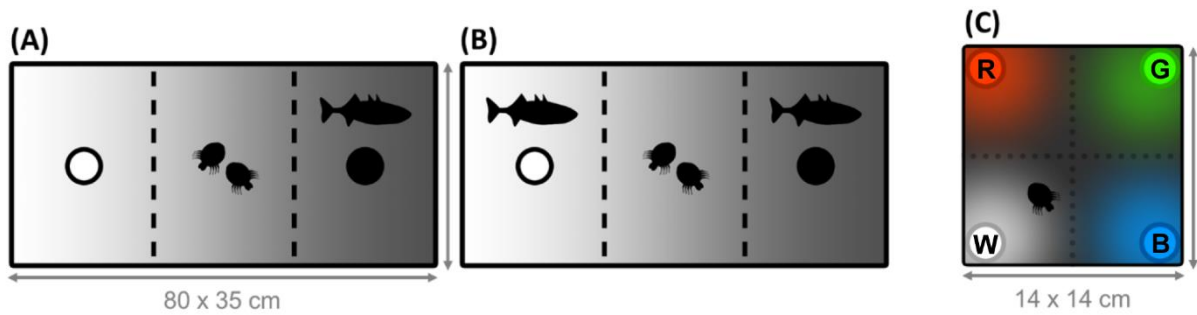
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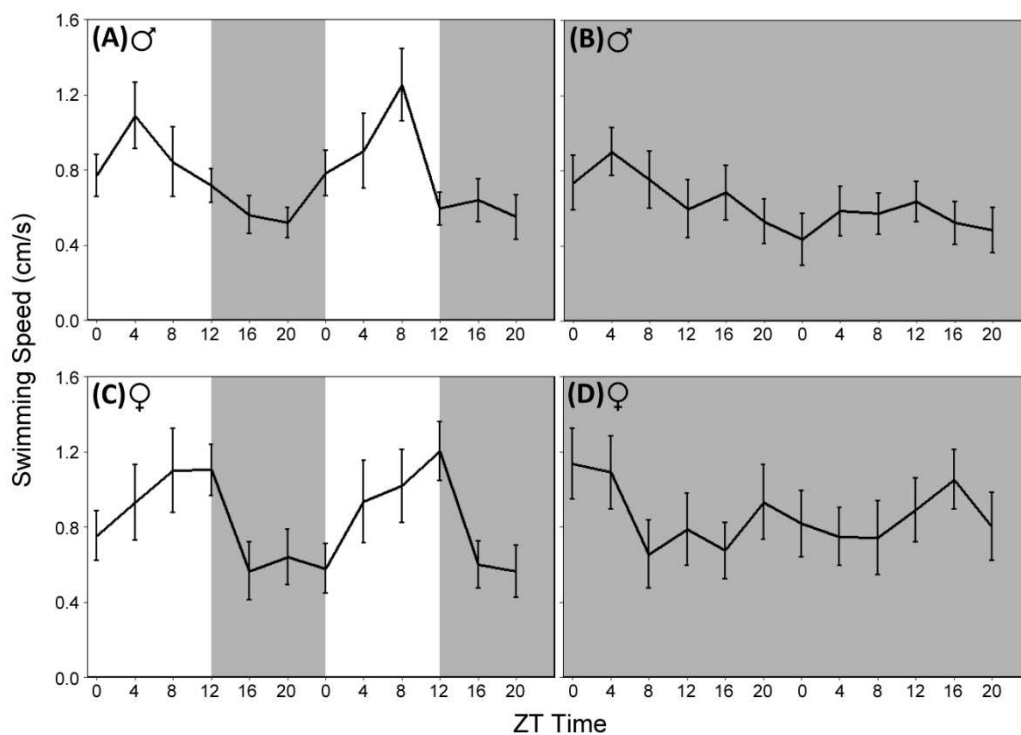
543 **Figures**



544

545 **Figure 1.** Plan view of experimental areas for *Argulus foliaceus* (A/B) light vs fish host
 546 preference and (C) light colour preference trials. In each arena circles represent LED light
 547 sources. (A) gives a choice of white light vs a three-spined stickleback (*Gasterosteus*
 548 *aculeatus*) host with a turned off light, (B) gives a choice of a white light + stickleback vs a
 549 turned off light + stickleback. In (A/B) dashed lines represent 1cm aperture mesh which allows
 550 the parasites to swim through while blocking fish movement. In (C) dotted lines indicate the
 551 total area of each coloured corner for behavioural recording, R = red light, G = green, W =
 552 white and B = blue (coloured light placement was changed/randomised for each trial).

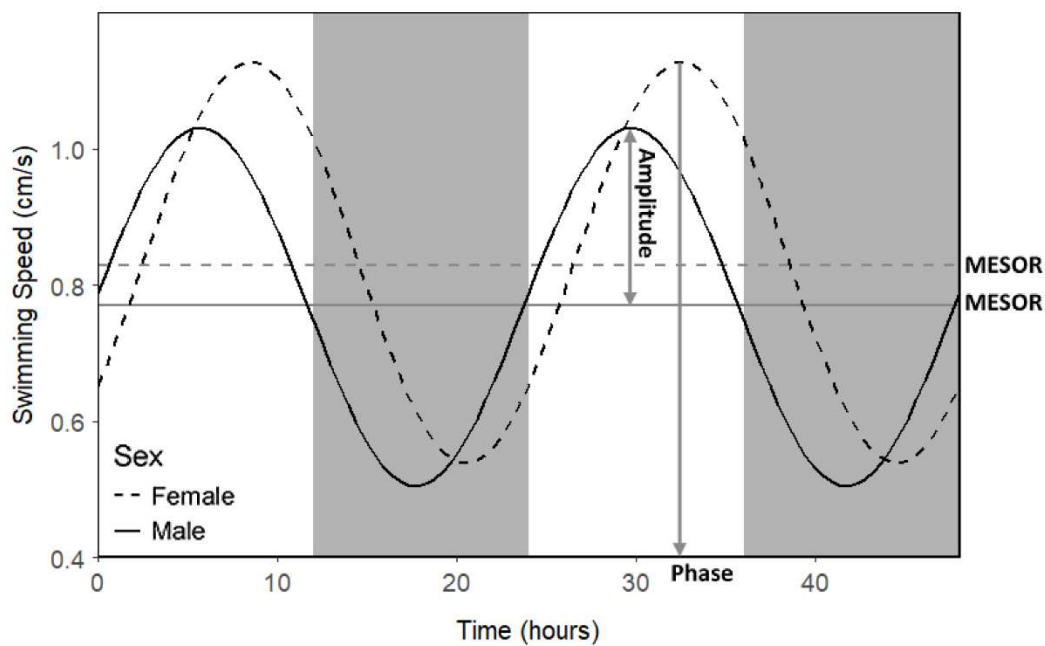
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554

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.

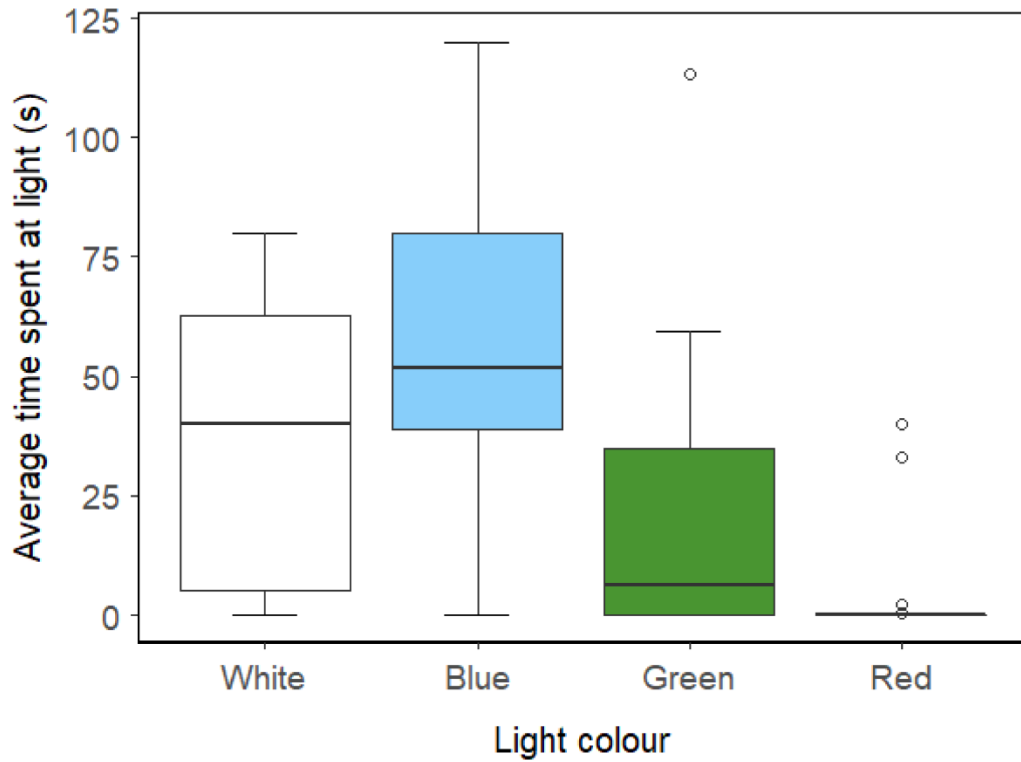
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563 **Figure 3.** Circacompore output plot of male and female *Argulus foliaceus* swimming speed
 564 over a 12:12 light:dark 48h period. Lights turn on/off at 0/12 and 24/36.

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566

567 **Figure 4.** Light preference of male *Argulus foliaceus* (n = 20) off the host. Average time spent
568 by free-swimming *A. foliaceus* in the vicinity of different coloured lights over a 2-minute
569 period. White light wavelength = 450-700 nm, blue = 450-490 nm, green = 520-560 nm, red =
570 635-700 nm.