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# 1 Life in the fast lane: temperature, density and host species impact survival and growth of 2 the fish ectoparasite *Argulus foliaceus*

3  
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## 7 8 **Abstract**

9 With expanding human populations, the food sector has faced constant pressure to  
10 sustainably expand and meet global production demands. In aquaculture this frequently  
11 manifests in an animal welfare crisis, with fish increasingly farmed under high production,  
12 high stress conditions. These intense environments can result in fish stocks having a high  
13 susceptibility to infection, with parasites and associated disease one of the main factors  
14 limiting industry growth. Prediction of infection dynamics is key to preventative treatment  
15 and mitigation. Considering the climatic and technology driven changes facing aquaculture,  
16 an understanding of how parasites react across a spectrum of conditions is required. Here we  
17 assessed the impact of temperature, infection density and host species on the life history  
18 traits of *Argulus foliaceus*, a common palearctic fish louse, representative of a parasite group  
19 problematic in freshwater aquaculture and fisheries worldwide. Temperature significantly  
20 affected development, growth and survival; parasites hatched and developed faster at higher  
21 temperatures, but also experienced shorter lifespans when maintained off the host. At high  
22 temperatures, these parasites will likely experience a short generation time as their life  
23 history traits are completed more rapidly. *A. foliaceus* additionally grew faster on natural  
24 hosts and at lower infection densities. Ultimately such results contribute to prediction of  
25 population dynamics, aiding development of effective control to improve animal welfare and  
26 reduce industry loss.

## 27 28 **Keywords**

29 Life history; Aquaculture; Climate change; Fish pathogen; Infectious disease

## 30 31 **1. Introduction**

32 Aquaculture has global economic benefits, providing food security and supplying stock for  
33 sport fishing and the ornamental pet trade (FAO 2018). As fisheries intensify to meet global  
34 demands, animals are subject to an increasing number of stressors (Wood 2001; Lewin *et al.*  
35 2006; Wedemeyer *et al.* 1997). Such conditions facilitate and amplify parasite transmission  
36 and disease outbreaks, with infections arguably the most important factor limiting  
37 sustainable industry expansion (Granada *et al.* 2016). Management of parasites and disease  
38 in fish is grossly lacking compared to mammalian species (Burka *et al.* 1997) with diagnosis  
39 and treatment difficult to accomplish, exacerbated by a lack of basic biological information  
40 coupled with high diversity in fishery cultures and structure (Li *et al.* 2002).

41 Fish farm infrastructure ranges from near natural systems to highly controlled artificial  
42 environments. Despite this diversity, all farms can experience temperature shifts due to  
43 climate change and/or increased use of technology (Jiang 2010). Temperature is crucial in  
44 farming, significantly influencing animal physiology and wellbeing. Associated parasites and  
45 disease are equally affected by temperature, which can lead to drastic shifts in infection  
46 dynamics. For parasites, high ambient temperatures typically lead to a short generation time  
47 as life history traits are completed more quickly; however, each trait can respond differently  
48 to temperature leading to trade-offs (e.g. Gophen 1976; Andersen and Buchmann 1998;  
49 Soleng *et al.* 1998; Sahoo *et al.* 2013). Examination of a suit of life history traits is therefore  
50 required to understand how temperature impacts overall parasite population dynamics. This  
51 is critical if we are to predict infection dynamics and develop more effective management  
52 practices.

53 One of the most conspicuous parasite groups plaguing fisheries are ectoparasitic fish  
54 lice, relatively large (compared to most fish parasites) crustaceans responsible for widespread  
55 damage in both marine and freshwater systems (Hakalahti *et al.* 2008; Costello 2009).  
56 Elevated temperatures are linked to outbreaks (Hakalahti *et al.* 2004a; Shimura 1983;  
57 Harrison *et al.* 2006), with modelling of marine sea lice showing a higher epidemic potential  
58 at higher temperatures (Groner *et al.* 2014). Freshwater lice (Genus *Argulus*), are particularly  
59 dependent on spring warming to induce hatching of overwintered eggs, which kick-start early  
60 population growth (Mikheev *et al.* 2001). As such, wild fisheries are predicted to encounter  
61 *Argulus* spp. earlier in the year and for prolonged periods under climate change scenarios,  
62 while in aquaculture systems maintained above 10 °C *Argulus* spp. can reproduce  
63 continuously (Hakalahti *et al.* 2006; Taylor *et al.* 2009a; Stewart *et al.* 2017). Freshwater lice  
64 are also a rising problem in UK angling fisheries; only one legal chemical treatment is currently  
65 available (Slice<sup>®</sup>, typically used against sea lice) with resistance a concern (Hakalahti *et al.*  
66 2004b; Taylor *et al.* 2005). Management strategies focusing on stocking practices can help  
67 reduce infection depending on application (McPherson *et al.* 2012), while control methods  
68 such as egg-laying traps lack testing (Taylor *et al.* 2005). To improve current application of  
69 management, an understanding of how *Argulus* spp. dynamics in fisheries change under  
70 differing environmental conditions is needed.

71 Here, we examined the impact of temperature on one of the most common Eurasian  
72 freshwater fish lice, *Argulus foliaceus* (see Radkhah 2017), before infection, during  
73 establishment and post-infection. Specifically, we aimed to identify changes in parasite  
74 hatching, growth and survival on and off the host. The impact of infection density and host  
75 species on parasite growth was also considered due to the variety of hosts across farming  
76 systems and tendency of *Argulus* to aggregate on the host. Temperature also has the  
77 potential to alter both of these factors by influencing host-parasite optima.

78

## 79 **2. Methods**

### 80 *2.1 Host origins and maintenance*

81 Three-spined sticklebacks (*Gasterosteus aculeatus*) were collected via hand netting from  
82 Roath Brook, Cardiff (ST 18897 78541) on 19/04/18 and 20/06/18, with ornamental guppies  
83 (*Poecilia reticulata*) purchased from a wholesaler on 29/06/18. Upon arrival at Cardiff  
84 University, all fish were lightly anaesthetised with 0.02% MS222 (tricaine methanesulfonate)  
85 and screened for ectoparasites using a dissection microscope with fibre optic illumination.  
86 Both species were infected with *Gyrodactylus* spp.; guppies were treated with Levamisole  
87 according to Schelkle *et al.* (2009) while for sticklebacks gyrodactylids were removed  
88 manually with watchmaker's forceps due to the low prevalence. No *Argulus* spp. were found  
89 on either fish species. All fish were acclimatised in a laboratory setting on a 12 h:12 h light:  
90 dark cycle, fed daily and maintained in stock tanks at a density <1 fish/L for 2 weeks prior to  
91 experimental use. Sticklebacks were maintained at  $14 \pm 0.5$  °C and fed *Tubifex* bloodworm,  
92 while guppies were kept at  $24 \pm 0.5$  °C and fed Aquarian® tropical fish flakes. Prior to  
93 experimental use, all fish were screened clear of ectoparasites three times (Schelkle *et al.*  
94 2009) and measured for standard and fork length (using callipers accurate to 0.1 mm).  
95 Throughout all experiments, location of *Argulus foliaceus* on the host was recorded to  
96 examine parasite movement.

97 Permission was obtained from local authorities prior to fish collection. All animal work  
98 was approved by the Cardiff University's Animal Ethics Committee, followed ARRIVE  
99 guidelines and was conducted under Home Office Licence PPL 303424.

100

## 101 2.2 Parasite cultures and infection

102 *Argulus foliaceus* were obtained from laboratory cultures, maintained using three-spined  
103 stickleback hosts (see Stewart *et al.* 2017). Two *A. foliaceus* strains were used in this study,  
104 lab strain (cultured 4 years in lab, origins detailed in Stewart *et al.* 2017) and wild strain  
105 (cultured 1 year in lab) originating from *A. foliaceus* adults (identified morphologically  
106 according to Fryer 1982) obtained from Rudd (*Scardinius erythrophthalmus*) from a fishery in  
107 Surrey on the 19<sup>th</sup> October 2017. To hatch *A. foliaceus* for experimental use, eggs laid in  
108 culture were removed from storage at 7°C (development ceases <10 °C; Shafir and As 1986)  
109 and gradually acclimated to incubation temperature (14 or 24 °C; experiment dependent) by  
110 placing them at ambient air temperature to allow gradual warming of the water (10 h to reach  
111 24 °C from 7 °C, 4 h to reach 14 °C). Temperatures were maintained using thermostatically  
112 controlled rooms; average temperature =  $24$  °C  $\pm$  0.52 SD and  $14$  °C  $\pm$  0.55 SD. Eggs were  
113 checked daily and given weekly water changes until hatching. No significant differences were  
114 found between lab and wild strain *A. foliaceus* regarding incubation time, hatching  
115 success/period and survival on/off the host (data not shown). As such, the most prolific  
116 culture was used at the time of each experiment (lab strain for hatching and survival  
117 experiments, wild strain for parasite growth experiments).

118 Infections were performed by placing a single fish into 100 ml water and introducing  
119 the required number of parasites via a pipette. In all cases, *Argulus* naturally attached to the  
120 fish within 10 min of exposure. For the hatching and survival experiments, time to infect was  
121 recorded for each parasite, however, no significance was found between temperature

122 treatments (shock, gradual or no temperature change;  $F_{3, 219} = 0.09$ ,  $p = 0.96$ ), with host length  
123 ( $F_{1, 218} = 0.01$ ,  $p = 0.92$ ) or with parasite position post infection ( $F_{12, 206} = 0.66$ ,  $p = 0.96$ ). To  
124 measure *A. foliaceus* on the host, infected fish were anaesthetised using 0.02 % MS-222 and  
125 placed under a dissecting microscope in a glass dish with 200 ml of dechlorinated water.  
126 Images were taken of attached *A. foliaceus* with fish laying flat on their sides, at 10x  
127 magnification using a Lumenera Infinity 1 camera with Infinity Capture software version 6.5.4.  
128 *A. foliaceus* were measured from the rostral tip of their carapace to caudal end of the  
129 abdominal lobes using ImageJ version 1.51j8 (Schneider *et al.* 2012). To measure *A. foliaceus*  
130 off the host, parasites were placed onto a slide using a pipette, restrained by reducing their  
131 pool of water to a minimal amount, and then imaged as above. All images were calibrated for  
132 measurements using a 1/100 mm micrometre scale.

133

### 134 2.3 Temperature impact on parasite hatching and survival

135 To determine the effect of temperature on *A. foliaceus* hatching success and survival, three  
136 temperature treatments were investigated: gradual temperature change (eggs incubated at  
137 24 °C with newly hatched parasites gradually cooled to 14 °C over 24 h), shock temperature  
138 change (eggs incubated at 24 °C with newly hatched parasites introduced to 14 °C water 24 h  
139 post hatching without acclimation) and finally no temperature change (eggs incubated and  
140 parasites maintained thereafter at 14 °C). For hatching success trials, three separate groups  
141 of eggs were incubated per temperature (14°C N = 132 eggs total, 24°C N = 476 eggs) with  
142 daily checks and weekly water changes.

143 For survival on the host, individual sticklebacks (average standard length = 40 mm ±  
144 0.45) were infected with five individuals of *A. foliaceus* (all from the same temperature  
145 treatment) 24 h post-hatching and placed into 1 L tanks at 14 °C (standard stickleback  
146 infection level; Stewart *et al.* 2017, N = 15 fish and N = 75 parasites per treatment). Any *A.*  
147 *foliaceus* lost during infection (N = 19 total) were presumed eaten and replaced (Bandilla *et*  
148 *al.* 2008). *A. foliaceus* survival was monitored on infected sticklebacks daily for 7 days and  
149 then weekly until 21 days post-infection.

150 Parasite survival off the host was assessed by placing newly hatched *A. foliaceus* into  
151 50 ml dechlorinated water at 14 °C (N = 30-65 parasites per treatment). Here an additional  
152 temperature treatment was tested with parasites hatched and maintained at 24 °C (N = 37  
153 parasites). *A. foliaceus* were monitored daily using a dissecting microscope with the number  
154 alive, moribund and dead recorded. Consistently, one day prior to death parasites were  
155 moribund - as this displayed the same trend as survival, it is not reported further.

156

### 157 2.4 Temperature, parasite density and host species impact on *A. foliaceus* growth

158 To ascertain the impact of temperature on *A. foliaceus* growth, sticklebacks acclimatised to  
159 14, 19 and 24 °C (1 week acclimation period) were infected with a single *A. foliaceus*  
160 metanauplius measured prior to infection (day 0, length = 0.618 mm ± 0.049 SD; N = 15 fish  
161 and parasites per temperature). To investigate any additional impact of host species and  
162 infection density on *A. foliaceus* growth, sticklebacks and ornamental guppies were selected

163 as two extremes. Sticklebacks are a temperate, natural host found in most waterbodies across  
164 the UK, versus guppies, a tropical fish and one of the most popular pet species with reports  
165 of *A. foliaceus* infection in aquaculture/pet trade (Walker *et al.* 2007; Momeni Shahraki *et al.*  
166 2014; Maceda-Veiga *et al.* 2016). For experimental work sticklebacks and guppies  
167 acclimatised to 19 °C were infected with one individual of *A. foliaceus* per 7.4 mm of host  
168 standard length (based on maximum non-lethal infection density of 5 parasites per  
169 stickleback: Stewart *et al.* 2017; N = 10 fish per host species, N = 3 - 5 parasites per fish). Post-  
170 infection, all fish were maintained individually in 1 L tanks with water changes every 48 h to  
171 maintain water quality. One day post-infection (day 1) and subsequently every 48 h for two  
172 weeks, *A. foliaceus* were measured on the host with their position noted. After 2 weeks, all *A.*  
173 *foliaceus* were removed from fish, sexed and re-measured off the host to give final parasite  
174 length.

175

## 176 2.5 Statistical Analysis

177 All statistical analyses were conducted in R statistical software v3.4.3 (R Core Team 2017)  
178 using the following packages: “ggplot2” to visualise the data (Wickham 2009), “survival” to  
179 run survival analyses (Therneau and Grambsch 2000; Therneau 2020) and “lme4” to run  
180 Generalised Linear Mixed Models (Bates *et al.* 2014). Models were refined through stepwise  
181 deletion of non-significant terms and Akaike information criterion comparisons, with visual  
182 examination of model plots to check standardised residuals for normal distribution and  
183 homogeneity of variance. In all mixed models fish ID was included as a random factor to  
184 account for pseudo-replication, and in all tests the level of significance was taken as  $p < 0.05$ .

185 To examine the survival of *A. foliaceus* on stickleback hosts, a Generalised Linear  
186 Mixed Model (GisedLMM) with Poisson family and square root link function was used with  
187 number of days post infection, temperature treatment (gradual, shock or none), an  
188 interaction between day and treatment and host standard length as dependent variables.  
189 Survival analysis was used to determine the effect of temperature treatment and time on *A.*  
190 *foliaceus* survival off the host. Hatching success of eggs was compared across treatments  
191 using a Chi-squared test.

192 A GisedLMM with Gaussian family and log link function was used to assess the impact  
193 of parasite sex, host standard length, host species, days post-infection, temperature and an  
194 interaction between day and temperature on *A. foliaceus* length. To analyse the impact of  
195 parasite density and host species on *A. foliaceus* length, two General Linear Mixed Models  
196 (GLMM) were used to examine the effect of days post-infection and host standard length,  
197 alongside either infection density and an interaction between infection density/day, or host  
198 species with an interaction between host species/day.

199 Additionally, for the growth experiments, a GisedLMM with binomial family and logit  
200 link function was used to assess whether parasite location (on the body of the host instead of  
201 the fins, yes/no) was affected by temperature, infection density, host species and time. To  
202 examine overall movement of *A. foliaceus* on hosts, a GisedLMM with binomial family and  
203 logit link function compared whether a parasite moved (yes/no) to temperature, host species,

infection density, days post-infection and host length. Two-Proportion Z-Tests were also used to compare the number of parasites on the body of the fish versus the fins across five parasite size groups: 0.40 - 0.79, 0.80 - 1.19, 1.20 - 1.59, 1.60 - 1.99 and 2.00 - 2.39 mm. These size ranges were based on *A. foliaceus* developmental stages (see Rushton-Mellor and Boxshall 1994), parasites larger than 2.4 mm length were not statistically assessed due to small sample size.

210

### 211 3. Results

#### 212 3.1 Temperature impact on parasite hatching and survival

213 At 24 °C, *Argulus foliaceus* eggs hatched after an average incubation period of 27 days (range  
214 19 – 39 days) while at 14 °C eggs hatched after 67 days (range 60 – 75 days). Hatching success  
215 of eggs across temperature treatment ranged from 57.7% to 63.7%, and success did not differ  
216 between eggs incubated at 24 vs 14 °C ( $\chi^2(1) = 2.36, p = 0.13$ ).

217 *A. foliaceus* maintained at 24 °C off the host had significantly lower survival than  
218 parasites maintained at 14 °C ( $\chi^2(3) = 54.10, p < 0.001$ , survival analysis; figure 1). On  
219 stickleback hosts, *A. foliaceus* survival still significantly decreased over time ( $F_{1, 420} = 67.02, p$   
220  $< 0.001$ , GLMM), with just under 50% survival 21 days post-infection (figure 1). Host length  
221 did not significantly impact parasite survival ( $F_{1, 417} = 2.63, p = 0.11$ , GisedLMM).

222 Survival of *A. foliaceus* on and off stickleback hosts was not impacted by incubation  
223 temperature (24 or 14 °C; off host  $\chi^2(1) = 1.60, p = 0.20$ , survival analysis, on host  $F_{2, 418} = 0.48,$   
224  $p = 0.78$ , GisedLMM) or temperature treatment post-hatching (gradual, shock or no thermal  
225 change; off host  $\chi^2(2) = 2.90, p = 0.23$ , survival analysis, on host  $F_{1, 419} = 0.90, p = 0.52,$   
226 GisedLMM).

227

#### 228 3.2 Temperature, parasite density and host species impact on *A. foliaceus* growth

229 *A. foliaceus* length increased with temperature and over time ( $F_{2, 360} = 104.96, p < 0.001,$   
230 GisedLMM; figure 2). At 14 days post-infection, *A. foliaceus* length averaged 2.5 mm at 24 °C,  
231 1.9 mm at 19 °C and 1.1 mm at 14 °C.

232 Parasite growth was significantly slower at high compared to low parasite density  
233 (figure 2;  $F_{1, 195} = 34.15, p < 0.001$ , GLMM). Parasite length was also affected by host species  
234 over time ( $F_{1, 156} = 11.69, p < 0.001$ , GLMM): when infected with multiple parasites, but at an  
235 equivalent density, sticklebacks had larger *A. foliaceus* than guppies.

236 Considering *A. foliaceus* averaged 0.618 mm length at birth, adulthood (4.7 mm; taken  
237 from Rushton-Mellor and Boxshall 1994; Taylor *et al.* 2009b) would take 124 days at 14 °C, 45  
238 days at 19 °C and 30 days at 24 °C for low infection stickleback hosts (assuming a linear growth  
239 pattern). For the higher infection density tests at 19°C, *A. foliaceus* would take 50 days to  
240 reach adulthood on sticklebacks and 55 days on guppies. These values are however an  
241 estimate as *Argulus* species do display diverse growth profiles (Rushton-Mellor and Boxshall  
242 1994; Pasternak *et al.* 2004), especially under natural, wild conditions (Taylor *et al.* 2009b).

243 In all tests, host length and parasite sex did not significantly impact *A. foliaceus* growth  
244 (host length;  $F_{1, 362} = 1.31, p = 0.24$ , parasite sex;  $F_{1, 363} = 3.14, p = 0.07$ , GLMM).

245

246 **3.3 Position and movement of *A. foliaceus* on hosts**

247 Significantly more *A. foliaceus* were found on the fins of hosts versus the body as temperature  
248 and time spent on host increased ( $F_{1, 822} = 4.60$ ,  $p = 0.041$  and  $F_{1, 823} = 6.86$ ,  $p = 0.008$   
249 respectively, GisedLMM). Parasite density and host species did not affect parasite position on  
250 host ( $F_{1, 822} = 0.01$ ,  $p = 0.71$  and  $F_{1, 821} = 0.21$ ,  $p = 0.65$  respectively, GisedLMM). *A. foliaceus*  
251 movement frequency was higher at high infection density and temperature ( $F_{1, 446} = 50.80$ ,  $p$   
252  $< 0.001$  and  $F_{1, 445} = 24.89$ ,  $p < 0.001$  respectively, GisedLMM), but was not affected by host  
253 species, time spent on host or host length ( $F_{1, 444} = 0.27$ ,  $p = 0.51$ ,  $F_{1, 446} = 1.43$ ,  $p = 0.05$  and  $F_{1,$   
254  $442} = 0.20$ ,  $p = 0.66$  respectively, GisedLMM). *A. foliaceus* position was also significantly  
255 influenced by parasite size; 70% of *A. foliaceus* 2.0 - 2.4 mm in length were located on the  
256 host's body, versus 47% of newly hatched parasites 0.4 - 0.8 mm length ( $\chi^2(1) = 4.36$ ,  $p =$   
257  $0.037$ , Two-Proportion Z-Test).

258

259 **4. Discussion**

260 Parasite generation time is intrinsically linked to environmental variables and here, *Argulus*  
261 *foliaceus* responded positively to increasing temperature with faster incubation and growth.  
262 However, life span off the host was reduced at higher temperatures, potentially impacting  
263 infection success. *A. foliaceus* also demonstrated a high resistance to sudden temperature  
264 changes, with a 10 °C temperature shock having no impact on parasite survival on or off the  
265 host. Infection density and host species also affected parasite growth, variables which could  
266 alter infection dynamic predictions especially as they can change drastically across  
267 temperature and farming system.

268 Both egg incubation and *A. foliaceus* growth on hosts were significantly faster at 24 °C  
269 compared to 14 °C, suggesting *A. foliaceus* at higher temperatures would experience a shorter  
270 generation time as birth and development occur more rapidly. While fast life history traits  
271 potentially allow parasites to rapidly exploit hosts, they may also limit infection depending on  
272 host density; for example in entomopathogenic nematodes, prolific availability of hosts  
273 benefits parasites with fast infection rates while limited host availability favours parasites  
274 with slower rates (Crossan *et al.* 2007). Nematodes with fast infection rates also had a  
275 corresponding trade off in fecundity and survival (Crossan *et al.* 2007), comparable to this  
276 study where *A. foliaceus* at 24 °C experienced fast incubation and growth, but also a  
277 significant reduction in survival off the host. High host densities (such as those encountered  
278 in aquaculture systems) could override this trade off, negating any impact of reduced survival  
279 at high temperatures. For angling fisheries however, replacement stocking to maintain low  
280 fish densities has been previously predicted to decrease parasite populations (McPherson *et*  
281 *al.* 2012). In this case parasite survival could potentially influence overall parasite population  
282 success, as survival at 14 °C was double compared to 24 °C allowing parasites at low  
283 temperatures more opportunities for infection. This low temperature survival was also  
284 double the observed survival in a previous report by Walker *et al.* (2011a) examining 1 day  
285 old *A. foliaceus* at 15 °C. This difference potentially arises from inter-population variation, the

286 1°C difference and/or the inclusion of aerators by Walker *et al.* (2011a), which could cause  
287 higher parasite activity and subsequent increased metabolic cost/shorter lifespan.

288 *A. foliaceus* were maintained at constant temperature under laboratory conditions,  
289 however in heterogenous environments the parasite could maximise survival by moving to  
290 thermally optimal areas. *A. foliaceus* have a thermal preference of 28 – 30 °C (Herter 1927,  
291 although shadows present in the experimental setup may have affected preference results;  
292 Lagerspetz and Vainio 2006) suggesting they do not select cooler temperatures (such as 14 °C)  
293 to increase off host survival. Regardless, the ability of any parasite to select preferential  
294 microclimates should be considered when examining infections in fisheries and aquaculture.

295 Another key factor contributing to the success of *A. foliaceus* is their broad host range;  
296 they are found on practically all fish species within their natural habitat, alongside successfully  
297 infecting novel, unnatural hosts including ornamentals such as goldfish, koi and guppies  
298 (Walker *et al.* 2007; Momeni Shahraki *et al.* 2014; Mirzaei and Khovand 2015). Despite a  
299 difference in growth rate, *A. foliaceus* successfully infected and survived on both natural,  
300 native stickleback hosts and novel guppy hosts. Possessing a broad host range allows parasites  
301 to exploit a wider host pool, providing an advantage over specialist parasites in systems with  
302 mixed host species (such as wild/angling fisheries). In comparison, monoculture aquaculture  
303 systems will likely give rise to specialist parasites as they outcompete generalists. Regardless  
304 of system, host availability should be considered when assessing problematic infections as  
305 both specialist (Pasternak *et al.* 2004) and generalist (shown here) *Argulus* species show  
306 differential growth across host species.

307 *Argulus* typically display an aggregated distribution within host populations (Bandilla  
308 *et al.* 2005; Walker *et al.* 2008). Here higher *A. foliaceus* density on the host resulted in lower  
309 parasite growth rate, as such parasite populations could display reduced growth as  
310 aggregation increases. Regarding parasite position on host, parasites increasingly moved from  
311 the host fins to the body as time progressed and at higher temperatures. *A. foliaceus* moved  
312 to the body when they reached 2.0 - 2.4 mm length (7<sup>th</sup> developmental stage, at which point  
313 the mouth-tube is fully developed; Rushton-Mellor and Boxshall 1994), indicating the start of  
314 blood feeding. Juveniles feed only on mucous/skin cells, whereas the blood-feeding adults  
315 cause greater host damage with the potential for secondary infections (Bower-Shore 1940;  
316 Bandilla *et al.* 2006; Walker *et al.* 2011b). Prioritising identification and treatment of *A.*  
317 *foliaceus* infections when parasites are <2 mm length (pre-blood feeding) would improve  
318 control by addressing infections before they cause significant damage, as currently (in UK  
319 angling fisheries) infections are primarily tackled once adults are present in high numbers.

320 Understanding the dynamics of parasites across their life cycle is critical for predicting  
321 changes to infection. *Argulus* populations have been previously modelled to examine the  
322 impact of control methods and predict population changes/generation time (Fenton *et al.*  
323 2006; Taylor *et al.* 2009b; McPherson *et al.* 2012; Kumar *et al.* 2017). Of these studies, Taylor  
324 *et al.* (2009b) used *A. foliaceus* length-frequency data to estimate egg incubation, hatching  
325 period and maturation rate of different cohorts within an angling fishery. The authors  
326 calculated a maturation time of 34 days at 24 °C, comparable to our estimate of 30 days with

327 the difference likely caused by variables such as host species and infection density (as shown  
328 here), and/or our use of a linear growth assumption for estimation. However, Taylor *et al.*  
329 (2009b) also observed an increase in hatching period with decreasing temperature (not  
330 observed here) suggesting that this trait may be governed by different seasonal variables such  
331 as light period (Bai 1981). Incorporating this new empirical data into existing and future  
332 models should therefore help verify outputs and improve modelling predictions. This in turn  
333 will help farmers select appropriate control methods and conditions to give the best trade-  
334 off for host growth and parasite restriction, reducing the impact of parasites on industry.

335

### 336 **Competing interests**

337 We declare that the authors have no competing interests. The views expressed here are those  
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339

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558

559 **Figure 1.** *Argulus foliaceus* survival off the host at 14 or 24 °C and on host (three-spined  
560 stickleback; *Gasterosteus aculeatus*) at 14 °C. For on host survival, all fish began with five  
561 metanauplii. Error bars represent 95% confidence intervals.

562

563 **Figure 2.** Impact of temperature, infection density and host species on *Argulus foliaceus*  
564 growth. Three-spined sticklebacks (*Gasterosteus aculeatus*) were infected with one individual  
565 *A. foliaceus* metanauplii at 14°C, 19 °C and 24 °C (infection density = low). Additional guppies  
566 (*Poecilia reticulata*) and sticklebacks at 19 °C were infected with one *A. foliaceus* metanauplii  
567 per 7.4 mm of host standard length (creating a starting infection number of 3-5 parasites per  
568 fish, infection density = high). Error bars represent 95% confidence intervals.