

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository:<https://orca.cardiff.ac.uk/id/eprint/115789/>

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

Citation for final published version:

Stewart, A, Hunt, Rhiannon, Mitchell, R, Muhawenimana, Valentine , Wilson, Christopher, Jackson, J and Cable, Joanne 2018. The cost of infection: *Argulus foliaceus* and its impact on the swimming performance of the three-spined stickleback (*Gasterosteus aculeatus*). *Interface* 15 (147) , 20180571.
10.1098/rsif.2018.0571

Publishers page: <http://dx.doi.org/10.1098/rsif.2018.0571>

Please note:

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

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



1 **The cost of infection: *Argulus foliaceus* and its impact on the swimming performance of**
2 **the three-spined stickleback (*Gasterosteus aculeatus*)**

3 Stewart, A^{1#}., Hunt, R¹., Mitchell, R¹., Muhawenimana, V²., Wilson, C. A. M. E.²., Jackson,
4 J³. A., Cable, J^{1*}.

5 ¹School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK.

6 ²School of Engineering, Cardiff University, Cardiff, CF24 3AA, UK.

7 ³School of Environment and Life Sciences, University of Salford, Salford, M5 4WX, UK.

8 # Current address: ¹Faculty of Health and Medical Sciences, University of Surrey, Surrey,
9 GU2 7XH, UK.

10 *Corresponding Author: cablej@cardiff.ac.uk

11 Keywords: Stickleback, *Gasterosteus aculeatus*, parasite, hydrodynamics, water flow, fish
12 behaviour.

13

14 **Abstract**

15 For fish, there can be multiple consequences of parasitic infections, including the physical
16 impacts on swimming and the pathological costs of infection. This study utilised the three-
17 spined stickleback (*Gasterosteus aculeatus*) and the ectoparasitic fish louse, *Argulus foliaceus*,
18 to assess both physical (including form drag and mass) and pathological effects of infection.
19 Both sustained (prolonged swimming within an open channel flume) and burst (C-start)
20 swimming performance were measured on individual fish before (Trials 1-2) and after infection
21 (Trials 3-5). Experimental infection occurred shortly before the third trial, when the physical
22 impacts of infection could be separated from any subsequent pathology as transmission of adult
23 parasites causes instantaneous drag effects prior to observable pathology. Despite the relatively
24 large size of the parasite and corresponding increase in hydrodynamic drag for the host, there
25 were no observable physical effects of infection on either sustained or burst host swimming. In
26 contrast, parasite-induced pathology is the most likely explanation for reduced swimming
27 performance across both tests. All sticklebacks displayed a preference for flow refugia,
28 swimming in low velocity regions of the flume, and this preference increased with both flow

29 rate and infection time. This study suggests that even with large, physically demanding
30 parasites their induced pathology is of greater concern than direct physical impact.

31 **Introduction**

32 Distinguishing whether parasites are directly or indirectly responsible for changes in host
33 performance, such as behaviour or energetic ability, is challenging. Observed changes may be
34 a direct result of infection or host manipulation, or simply a consequence of host damage during
35 infection (1). When examining the impacts of parasite infection, most studies focus on the
36 pathological aspects of infection, which include a reduction in available nutrients due to
37 parasite feeding (2), cytokine driven sickness (3), injected or secreted toxins (4), physical tissue
38 damage either directly from the parasite or indirectly via inflammation (5), and/or the
39 redistribution of resources such as upregulation of the immune response (6). The indirect,
40 physical aspects of parasites are often not addressed, despite their conspicuous appearance as
41 changes in host shape and size. Host mobility in particular may be hindered by large or heavy
42 parasites, exacerbated by their positioning on the host. For fish, this could impact their
43 streamlined profile by increasing hydrodynamic drag and factors such as total mass or mass
44 distribution, causing an imbalance in stability. Infected fish may also exhibit energetically
45 costly 'flashing' or 'twisting' behaviour whereby the fish rubs up against hard substrates or
46 violently summersaults in an attempt to dislodge parasites (7). In contrast, the pathological
47 impacts of infection are often harder to discern.

48 The different impacts of fish parasites on their hosts have been studied extensively (8). The
49 cestode *Schistocephalus solidus*, for example, alters host shoaling swimming behaviour and
50 anti-predator avoidance to improve its transmission (9-16), as well as decrementing host
51 energetics and nutrition (17, 18). But even for this well-studied parasite, it is unclear whether
52 these alterations are directly or indirectly caused by the parasite (19). Economically, sea lice
53 are the most important large ectoparasite of fish. Sub-lethal infections with these lice reduces
54 Atlantic salmon swimming performance 4-5 weeks post infection (20). The ability to dissociate
55 whether this impact is due to physical and/or pathological effects is however difficult,
56 particularly with long-term infections. Additionally, the highly pathogenic nature of sea lice
57 results in haemorrhaging and widespread damage to the epidermis (21, 22) masking the
58 physical effects of infection. Similarly, Östlund-Nilsson, Curtis (23) assessed the physiological
59 impacts of infection with *Anilocra apongonae* (another large ectoparasitic crustacean) on
60 *Cheilodipterus quinquelineatus* and although they suggested that reduced host swimming

61 ability was caused by increased drag, this was not tested experimentally, thus the effects of
62 pathology and mechanical drag were not disentangled.

63 At a physical level, the drag on standard objects such as cylinders and aerofoils is well
64 understood (24), but few such studies have been performed on fish given the complex and
65 highly varied nature of their profile, with some exceptions including shark skin where the
66 structure of the denticles has been reverse engineered (25). If a parasite is large relative to fish
67 body size the streamlined hydrofoil of a fish is likely to be compromised, increasing form drag
68 and altering swimming performance. An estimate of the likely increase in hydrodynamic drag
69 due to parasite attachment can be calculated using the classical drag force formula:

$$70 \quad F = \frac{1}{2} C_D \rho U_0^2 A$$

71 where F is the drag force, C_D the drag coefficient which is a function of the Reynolds number
72 and body profile, ρ the fluid density, U the velocity and A is the frontal projected area of the
73 body (24). Although the relative change in the drag coefficient is unknown, an approximate
74 estimate of the increase in drag force (hereafter simply referred to as drag) can be calculated
75 based solely on the increase in the frontal projected area of the fish with the parasite attached
76 to its body. Furthermore, as external tagging affects fish swimming stability and ability to
77 remain parallel to the bed, parasites could also alter fish swimming performance (26, 27). A
78 parasite attached to the tail of the fish will therefore not increase projected area but may have
79 an impact on buoyancy and stability.

80 We undertook the current study to partition the physical and pathological impacts of infection
81 on fish swimming performance and examine how infection detrimentally impacts fish
82 swimming and predator avoidance. We used the freshwater fish louse *Argulus foliaceus* (total
83 length of 3-7 mm) infecting three-spined sticklebacks *Gasterosteus aculeatus* (typically 30-50
84 mm standard length at adulthood in the UK) as our model system. Argulids are the freshwater
85 equivalent of sea lice, but also a major problem in their own right (28). Individual *A. foliaceus*
86 occupy a relatively large area of this fish and can be directly transmitted as adults among hosts,
87 making this an ideal model for maximising physical effects while also reducing the
88 confounding effect of pathology. The parasite though is a generalist known to infect a large
89 number of commercially important fish with moderate pathological effects over time at low
90 infection intensities (28-31). These include localised inflammation and mechanical damage
91 from the spines and the stylet feeding mechanism, anaemia, weight loss and scale loss, which

92 cause lethargy or erratic behaviour (31). Specifically, we compared sustained and burst
93 swimming ability of hosts before infection, shortly after infection (when confounding factors
94 such as pathology would be negligible and any disruption of host swimming could be attributed
95 to the direct physical effects of the parasite), and several days after infection (to assess the
96 pathological effects of infection).

97

98 **Materials and Methods**

99 *Fish and parasite origin*

100 Three-spined sticklebacks (*Gasterosteus aculeatus*) were initially collected from an *Argulus*
101 naïve population caught in Roath Brook, Cardiff (ST 18897 78541) on the 2nd July 2015 and
102 transported to the aquarium facility at Cardiff University. Fish (mean standard length = 31.5
103 mm, range = 26.1 to 37.3 mm; mean mass = 0.471 g, range = 0.249 to 0.655 g) were maintained
104 in 30 L tanks at 15°C at a density <1 fish/L on a 18 h light: 6 h dark cycle and fed daily on
105 frozen chironomid larvae. Prior to performance tests, fish were treated for ectoparasites by
106 submersion in 0.004% formaldehyde solution for 1 h with a 30 min rest period in freshwater
107 after 30 min (see 32). These wild caught fish had a low to moderate incidence of *Gyrodactylus*
108 *gasterostei* as per previous surveys of this population (33, 34). Fish were then maintained in
109 1% salt solution with 0.002 g/L of methylene blue for 48 h to inhibit secondary infection.
110 Treated fish were checked visually for ectoparasites at least three times under a dissection
111 microscope with fibre optic illumination by anaesthetising them in 0.02% w/v MS222. Any
112 remaining ectoparasites were removed with watchmaker's forceps following the methods of
113 Schelkle, Shinn (35). Any fish found to have ectoparasites were checked a further three times
114 to ensure clearance of infection. Sticklebacks were then maintained for 2 weeks prior to swim
115 performance tests to allow recovery in dechlorinated freshwater. *Argulus foliaceus* were
116 obtained from a lab culture using three-spined sticklebacks, see Stewart, Jackson (32), bred
117 from specimens originally obtained from a carp (*Cyprinus carpio*) still water fishery in North
118 Lincolnshire, July 2014. Briefly, one juvenile female was raised to adulthood in isolation and
119 mated with one male, all offspring were descendants of this pairing. All animal work was
120 approved by the Cardiff University's Animal Ethics Committee and conducted under Home
121 Office Licence PPL 302357.

122

123 *Experimental design*

124 A total of five sustained swimming performance tests (see below), each separated by three
125 days, were performed on each fish with the first two tests acting as controls and allowing the
126 fish to acclimatise to trials in the flume (designated trial 1 and 2). The third performance test
127 (trial 3) was conducted a maximum of 30 min after infection with *A. foliaceus* (mean mass =
128 0.08 g, range = 0.05-0.13 g). All *A. foliaceus* used were full-sized adults to negate the effect of
129 parasite growth during the experiment and to maximise physical impacts. Infection was
130 conducted by exposing fish to two individuals of *A. foliaceus* in 100 ml of water (n=8) with the
131 controls handled in the same manner but not infected (n=5). All individuals of *A. foliaceus* had
132 been starved for 48 h prior to infection to facilitate natural attachment without the use of
133 anaesthetics, infection success was 100%. Fish were kept individually in 1 L tanks to avoid
134 cross infection. Infection was then monitored over the course of the trial and detached parasites
135 allowed to reattach again in 100 ml of water. In cases where *Argulus* or fish died or were
136 euthanized prior to the end of the experiment, their data was removed and not reported here.
137 The remaining two trials (3 and 6 days post-infection, trials 4 and 5) were used to measure the
138 effects of pathology on swimming performance. Across all infected and uninfected fish a total
139 of 65 sustained distance performance tests were performed. Burst swimming (C-start)
140 responses of each fish were additionally recorded 24 h after each sustained distance flume run
141 (as below). After all trials had been conducted the fish were euthanized in 0.002% MS222 and
142 standard length, pectoral fin length, caudal fin width and length, mass, sex and gravidity
143 recorded.

144 Swimming ability was measured in two ways: ‘sustained swimming’ in a flume where a fish
145 must swim against an increasing current until it is exhausted and their antipredator escape
146 (burst swimming) response. Depending on the species of fish anti-predator responses are
147 characterised by the shape the fish makes in the first few milliseconds of the escape, commonly
148 a ‘C’ or an ‘S’ shape (36, 37). The velocity of this C-start response in sticklebacks is
149 proportional to the likelihood of escape and is therefore a good measure of relative host survival
150 (38, 39).

151 *The Flume*

152 Sustained swimming performance tests were conducted in a unidirectional recirculating open
153 channel Armfield C4 multi-purpose flume (4 m length, 76 mm width and 150 mm depth) set
154 with a negative bed gradient of 1/1000. A weir gate at the downstream end of the flume was
155 used to control the longitudinal water surface profile and the flow depth was set at 105 mm.
156 Two 20 mm lengths of honeycomb flow straightener were used to contain fish within a 1 m

157 length of the flume (Fig. 1). Swimming performance tests were conducted during daylight
158 hours and water temperature was maintained at 22.9°C (SE±0.18) using ice blocks in the
159 reservoir to counteract the effects of heating from the pump and the non-temperature controlled
160 room. Haloex chloride treatment was used at 0.02 ml/L to remove chlorides and additional air
161 bubbled into the flume reservoir using a mains operated stone aerator. A 20 mm² measurement
162 grid was placed along the back sidewall of the flume to facilitate behavioural observations.

163 *Sustained distance swim performance test*

164 Each stickleback was placed into the flume while it ran at 0 L/s for 5 min of acclimatisation.
165 The flow rate was then increased every 5 min to 0.4, 0.7, 1.0, 1.3, 1.6, 1.9, 2.2 to a maximum
166 of 2.5 L/s at which fish were maintained for 20 min or until fish exhausted. Fish were
167 considered exhausted when pushed up against the downstream flow straightener and the time
168 till exhaustion used as a measure of sustained swimming performance. Fish were recorded
169 using a Swann DVR8-3425 960H resolution CCTV system. The videos for trials 2, 3 and 5
170 were analysed in JWatcher 0.9 (40) for time spent in the four separate regions of the flume over
171 each trial (Fig. 1) and assessed for five different behaviours: being pushed backwards
172 (movement downstream but while facing upstream), swimming downstream, station holding
173 (head maintained in the same 20 mm² space of the flume; see Gerstner and Webb (41),
174 swimming upstream and a twisting or flashing behaviour that appeared to attempts to dislodge
175 *A. foliaceus*. In addition, photographs of the anterior/medial (head on) view of each fish were
176 taken using a Nikon S3600 with a ruler in the frame of reference. These images were imported
177 into ImageJ (42) to calculate the frontal projected area of the fish with (e.g. Fig. 3C in 32) and
178 without parasites using the freehand selection tool. 'Projected area increase' was calculated as
179 the percentage increase in area for a fish with a parasite on a trial by trial basis and used as a
180 proxy for 'drag force'.

181 For behavioural observations, the flume was divided into four zones based predominantly on
182 flume velocity distributions but also on observations of sticklebacks in a preliminary trial,
183 demonstrating a preference for Zone-3 (Fig. 1 and Appendix 1). Flume velocities were
184 measured using a Nixon propeller meter with a sampling time of 3 min at 20 mm horizontal
185 and vertical intervals along the centreline of the flume. Velocity profiles with longitudinal
186 distance along the flume and for the zones are shown for the flow rate of 1.6 L/s in Appendix
187 1. In the near-bed zone ($Y \leq 1.5$ cm), velocities decreased with increasing longitudinal distance
188 from the upstream boundary (Appendix 1A). The near-bed zone in the centre of the control
189 volume (Zone-3) had slightly higher velocities than at the upstream boundary in Zone-4 (see

190 Fig. 2B) but did not statistically differ from one another (Appendix 2); determined by a linear
191 model with velocity (cm/s) as the dependent variable and flowrate (L/s) and zone as
192 independent variables including an interaction between the two independent variables. As
193 would be expected, the velocities were higher in the upper part of the water column (Zone-1)
194 away from the near-bed region (Zone-3 and 4; $p < 0.001$), while the flow accelerates and the
195 velocities are highest in the zone closest to the downstream boundary (Zone-2), which had a
196 significantly ($p < 0.001$) higher velocity than the remainder of the flume (Appendix 1B and 2).

197

198 *C-start performance test*

199 The C-start response of each fish was conducted in a 300 x 400 mm glass experimental arena
200 filled with dechlorinated water to a depth of 30 mm, allowing fish to move only along a
201 horizontal plane. A Nikon D3200 camera was used to film each trial at a frame rate of 50 fps.
202 Upon introduction to the tank fish were acclimatised for 5 min. A net was then thrust into the
203 water of the tank 5-10 cm from the head of the fish in order to initiate the response; a 2 min
204 recovery period was allowed and three trials of C-start conducted (43-45). A frame-by-frame
205 analysis was performed in Tracker v4.87 (46) with the velocity of the C-start calculated from
206 the 20 ms preceding initiation of the response; an average of the three C-start velocities was
207 then taken. The same sticklebacks were used in the C-start responses as in the sustained flume
208 tests, with C-start tests occurring 24 h after each flume trial.

209

210 *Statistical analysis*

211 All data were analysed using R v3.2.2 (47) with the additional use of ‘car’ (48), ‘MASS’ (49),
212 ‘lme4’ (50), ‘lmerTest’ (51) and ‘ggplot2’ (52) packages. All model selection was conducted
213 using Akaike Information Criterion. Least-squared means were used to compare within any 2-
214 way factorial interactions. Random terms were tested for using a likelihood ratio test. For
215 clarity, ‘infection group’ refers to the treatment fish were exposed to (a fish in the infected
216 treatment group would therefore be uninfected at trials 1 and 2) and ‘infection status’ refers to
217 the actual presence or absence of an infection at any given time.

218 To assess the effect of infection on swimming ability (sustained swimming and c-start) Linear
219 Mixed effects Models (LMMs) were used for the assessment of sustained and burst (C-start)
220 swimming performance with fish identification used as the random factor and the independent
221 variables: trial, infection group, 'trial: infection group', temperature, fish body condition
222 (residuals from a regression of mass and length³), sex, fish length, caudal fin size (principal
223 component of fin width and length) and pectoral fin size (fin length). Sustained swimming
224 ability was analysed using time spent in the flume as a proportion of the total possible time (55
225 min – not including acclimatisation) as the dependant variable with a logit transformation. C-
226 start performance used the mean velocity within the first 20 ms of the escape response from
227 three repeats within each trial as the dependant variable, with a square root transformation. A
228 further LMM was used to look for an effect of drag on sustained swimming ability; this analysis
229 utilised an adjusted version of the sustained swimming ability model with 'projected area
230 increase' used in place of the 'infection group' and limited to trials 2 and 3 with no interaction
231 (data was limited to trials 2 and 3 to remove the confounding impact of pathology).

232 The preference of fish for certain flume regions was analysed using a Chi-squared test with the
233 observed as the proportional length of time fish spent in a given zone and the expected as the
234 relative size of the flume zone (Ratio = Z1(0.784):Z2(0.02):Z3(0.012):Z4(0.184)). Further
235 LMMs tested which variables altered fish preference for flume zones. Individual models for
236 each flume zone (to avoid autocorrelation) were used with logit transformed proportional time
237 as the dependant variable (trials 2, 3 and 5) and the independent variables: flow rate, trial,
238 infection status, length, condition, sex, 'trial: Infection status' and 'flow rate: infection status'
239 with fish identification as a random factor. To confirm the effect of trial on these models as
240 fish only had a positive infection status from trial 3 onwards, further LMMs were run using
241 'infection group' (comparing the control group to experimental group) in place of 'infection
242 status' (comparing infected individuals to all controls).

243 The effect of fish positioning in the flume on sustained swimming performance was analysed
244 using trials 2, 3 and 5. This positional analysis used four models that comprised the minimal
245 model from the 'sustained swimming performance' analysis (ProportionalTime ~
246 Trial*InfectionGroup) with the addition of the proportion of time spent in each of the flume
247 zones as an independent variable (proportional time in each zone was used to account for bias
248 caused by fish swimming for different time periods). An interaction between each of the flume

249 zones and the infection group was also tested but had no impact on the models. Each of these
250 four models were then compared to the minimal model using a deletion test.

251 Stickleback swimming behaviour was analysed using individual linear mixed models for each
252 behaviour, with the dependant variable as proportion of time each fish spent performing a
253 behaviour (logit transformed) and fish identification as the random variable. Additional
254 independent variables included the fish behaviour, flow rate (L/s), infection status, temperature
255 and a 'flow rate: infection status' interaction. Argulid removal behaviours, flashing or twisting
256 in order to dislodge the parasite (7), were not analysed as only a few individuals exhibited this
257 behaviour and for very short time periods.

258 **Results**

259 *Impact of Argulus on host profile*

260 The mean projected area for three-spined sticklebacks (*Gasterosteus aculeatus*) infected with
261 two individuals of *Argulus foliaceus* increased by 8.4%. When considering only fish with one
262 or both *A. foliaceus* individuals attached to the head (47% of infected fish in this study), the
263 projected area increased on average by 15.3% (range: 9.7-26.5%). For fish with both *A.*
264 *foliaceus* located on the body (53% of infected fish), the projected area did not increase.
265 However, individual *A. foliaceus* were motile between trials, the average change in host
266 projected area between trials was 7.4%.

267 *Effect of infection on sustained and burst swimming ability*

268 Sticklebacks infected with *A. foliaceus* for 6 days demonstrated a significant reduction in
269 sustained swimming performance (Fig. 2A). Among infected fish there was a significant drop
270 in swimming performance between control trials and later trials 4 and 5 indicating an effect of
271 pathology, while no effect of parasite presence was observed in earlier trials (Table 1). When
272 comparing the uninfected group to the infected, trials 4 (t.ratio=2.208, $p=0.032$) and 5
273 (t.ratio=3.172, $p=0.003$) differed significantly (Fig. 2A). The burst swimming of these same
274 infected sticklebacks had also reduced significantly by trials 4 and 5, but not at other time
275 points (Fig. 2B). Among uninfected fish there were no significant differences between
276 sustained or c-start tests and independent factors (temperature, flume side, fish length,
277 condition, sex, pectoral/caudal fin size) had no effects on the models, but individual fish
278 behaviour was discrete (significant fish identification $p=0.01$).

279 *Fish preferences for flume zones*

280 Sticklebacks demonstrated a preference for swimming in Zone-3 (upstream near-bed boundary;
 281 $\chi^2=16.750$, $p<0.001$) but no other zones ($p>0.05$). Sticklebacks also had an increasing
 282 preference for Zone-3 across five trials in higher flow rate conditions for both infected and
 283 uninfected fish ($t=10.011$, $df=28$, $p<0.001$; Fig. 3A) and this increase in preference was
 284 stronger in the infected fish ($t=2.829$, $p=0.005$; Fig. 3A). For infected fish, there was an increase
 285 in time spent in Zone-2 in later trials as they exhausted more quickly ($t\text{-value}=3.632$, $df=227$,
 286 $p <0.001$; Fig. 3B), while on average all fish spent less time in this zone with increasing flow
 287 rate ($t\text{-value}=-6.633$, $df=21$, $p <0.001$). There was also a drop in fish spending time in Zone-1
 288 (relatively high velocity zone) correlated with the increasing time spent in other zones at higher
 289 flow rates ($t\text{-value}=-10.417$, $df=226$, $p<0.001$) and larger fish spent more time in Zone-2 ($t\text{-}$
 290 $value=2.474$, $df=9.176$, $p=0.035$). Analysis of swimming position in the flume revealed fish
 291 which spent longer in Zone-3 were able to swim for a proportionally longer time ($t\text{-}$
 292 $value=4.147$, $df=26$, $p<0.001$). In all cases, fish identification had a significant effect on the
 293 model ($p<0.05$).

294 *Behaviour*

295 Overall, fish performed more station holding ($\chi^2=0.707$, $p<0.05$) than other behaviours
 296 ($p>0.05$). With increasing flow rate more fish performed station holding ($t=4.070$, $df=228$,
 297 $p<0.001$; Fig. 4) and infected fish spent more time holding station in the flume than uninfected
 298 fish ($t=2.862$, $df=232$, $p=0.005$; Fig. 4), although there was no interaction between the two.
 299 These infected fish also had a corresponding drop in time spent swimming upstream at higher
 300 flow rates ($t=-2.882$, $df=228$, $p=0.004$). Sticklebacks also decreased the proportion of time
 301 spent swimming upstream in higher flow rates ($t=-3.962$, $df=228$, $p<0.001$). In all cases, fish
 302 identification had a significant effect on the models ($p<0.05$).

303 **Discussion**

304 Using sticklebacks infected with *Argulus foliaceus* in both sustained distance and C-start burst
 305 swimming, we found that *A. foliaceus* pathology had a significant negative impact on both
 306 forms of swimming. The lack of swimming performance reduction in the third trial performed
 307 immediately post-infection, compared with the first two pre-infection trials and the uninfected
 308 fish, suggests that there was no impact of infection on hydrodynamic drag (no effect of
 309 projected area) or instability (resulting from increased additional and uneven mass i.e. no effect
 310 of parasite presence) on fish swimming performance.

311 In comparison to external fish tags, (26, 27) and the previous suggestions that drag from isopod
312 infections (23) contribute to poor swimming performance, no effect of hydrodynamic drag or
313 instability was observed in either swimming test in the current study. This is despite the
314 parasites increasing the projected area of the fish by as much as 26.5% (mean 15.3%). For
315 comparison, with external tagging the increase in drag force is estimated to be 12-13% for 47-
316 72 cm cod with tags of 1.87 and 4.15 cm² frontal area (53). The streamlined profile of *A.*
317 *foliaceus*, holding itself close to the fish's body, could explain the lack of drag and mass effects;
318 we also checked to see if neutral buoyancy might be a possible explanation but *A. foliaceus*
319 sink at a rate of 4.6 mm/s in a 10 ml glass measuring cylinder. It is also possible that a larger
320 projected area increase is required to observe these effects in the laboratory, but such high
321 intensity aggregated infections towards the head are unlikely in nature (54). Additionally,
322 sticklebacks may be able to compensate for increased drag or instability during the early stages
323 of infection (when only physical consequences are present), masking the physical effects of
324 infection. The direct life cycle of *A. foliaceus* with no intermediate host means that if the host
325 fish is consumed then the parasite's germline will also be lost, suggesting that rapid
326 deterioration of the host is not evolutionarily favourable in this instance. A high impact on fish
327 physiology is therefore best avoided, at least until the parasite has fed and bred.

328 The continued presence of *A. foliaceus* is likely to compound the pathological effect on
329 swimming performance, with a continued reduction in swimming performance from the point
330 of infection. This was demonstrated by the greater magnitude of performance reduction at 6
331 days post-infection compared to 0 or 3 days post-infection. This reduction is likely derived
332 from the feeding and attachment mechanisms of the argulid, which is reliant on blood feeding
333 by means of a stylet and cytolytic toxins with attachment by large maxillae suckers and
334 numerous spines on the ventral surface (55-57). These two mechanisms can cause necrosis and
335 apoptosis (58-60), either directly or via inflammation, and are likely to be a major cause of fish
336 swimming performance reduction reducing the fish's overall health; particularly when
337 immune-pathological costs such as cytokine driven sickness and nutrient redistribution are also
338 taken into account. Fish infected with large parasites, such as isopods, also have increased
339 oxygen consumption and a higher fin beating frequency which may contribute to pathology
340 and reduce swimming performance (23); such effects may only be observable sometime after
341 infection when the increased metabolism has used up stored nutrients. A fish in the wild on a
342 lower calorie intake than within lab conditions may therefore experience a greater detrimental
343 effect of infection. Such fish would likely have increased swimming stresses resulting in a

344 positive pathological feedback loop that increases susceptibility to predators and detrimentally
345 impacts feeding, swimming and mating.

346 Although the flow depth was relatively constant along the longitudinal axis of the flume, there
347 was some variation in the velocity due to the flow straighteners and short length of the flume.
348 The velocity also varied transversely due to the side walls and with vertical distance from the
349 bed. Along the bed and sides of an open channel flume, the velocity is reduced due to boundary
350 friction and the velocity gradient is higher in these zones. Multiple studies have demonstrated
351 that fish use this boundary layer as a shelter from higher velocities allowing them to attain
352 higher swim performance (41, 61, 62). The current study also observed a bias in fish behaviour
353 towards swimming in this lower velocity region of the flume, in a process known as flow
354 refuging (63). The preference of sticklebacks for this low velocity zone was further enhanced
355 in increasing flow rate as previously found by Barbin and Krueger (61) in American eels
356 (*Anguilla rostrata*). Fish infected with *A. foliaceus* demonstrated an even greater preference
357 for this same low velocity region than their uninfected counterparts, as previously reported by
358 Hockley, Wilson (64). In addition to the energy saving behaviours observed around the
359 boundary layer, infected fish also spent a greater proportion of their time swimming in a static
360 position in the flume and not swimming up or down its 1 m length. With the combined
361 preference for low velocity, low energy swimming infected sticklebacks appear to be
362 demonstrating heightened energy saving behaviours in order to offset the negative impacts of
363 infection on swimming performance. Such a response could be comparable to fish or other
364 animals that become less active when infected with certain parasite taxa (65, 66) as pro-
365 inflammatory cytokines drive lethargy and sickness behaviours. Additionally, we found that
366 fish with larger pectoral fins spent more time holding station. This particular station holding
367 behaviour typically involves labriform locomotion (67, 68), which is less energetic than the
368 subcarangiform locomotion also displayed by sticklebacks, indicating larger finned fish may
369 be using this form of locomotion as a more energy efficient swimming technique given that
370 efficiency of this swimming is related to pectoral fin size (69, 70).

371 In summary, this study has revealed a major impact of parasite-induced pathology on fish
372 swimming performance, but a perhaps surprising lack of hydrodynamic effect caused by
373 increased drag or instability due to the relatively bulky *A. foliaceus* infection. Sticklebacks also
374 showed a strong preference for low velocity regions of the flume and for energy saving
375 behaviours, particularly at higher flow rates or when infected. Lastly, fish with larger pectoral
376 fins spend more time performing stationary swimming using labriform locomotion, also

377 attributed to energy saving and the fact that at higher velocities larger fins will give greater
 378 thrust. Despite the size of the *A. foliaceus* ectoparasites causing significant increases to
 379 projected host area and corresponding increases in the hydrodynamic drag, the pathological
 380 effects are of greater consequence to the fish and result in a shift in fish swimming towards
 381 energy saving behaviours.

382 **Acknowledgements**

383 This work was funded by a research grant from the Leverhulme Trust (RPG-301) and a NERC
 384 GW4+ PhD studentship to RH (NE/L002434/1). We thank three anonymous referees for their
 385 comments on an earlier version of this manuscript.

386 **Author contribution:** JC and AS conceived and designed the study; VM and CAME provided
 387 advice on experimental design; AS, RH, RM and VM performed the experiments; JC and AS
 388 drafted the MS, which was commented on by all authors.

389 **References**

- 390 1. Poulin R. 1995 “Adaptive” changes in the behaviour of parasitized animals: A critical review.
 391 Int J Parasitol. 25: 1371-83. [http://dx.doi.org/10.1016/0020-7519\(95\)00100-X](http://dx.doi.org/10.1016/0020-7519(95)00100-X).
- 392 2. Zuzarte-Luís V, Mota MM. 2018 Parasite Sensing of Host Nutrients and Environmental Cues.
 393 Cell Host Microbe. 23: 749-58. <https://doi.org/10.1016/j.chom.2018.05.018>.
- 394 3. Clark IA, Budd AC, Alleva LM, Cowden WB. 2006 Human malarial disease: a consequence
 395 of inflammatory cytokine release. Malar J 5: 85. <http://doi.org/10.1186/1475-2875-5-85>.
- 396 4. Carpio A, Romo ML, Parkhouse RME, Short B, Dua T. 2016 Parasitic diseases of the central
 397 nervous system: lessons for clinicians and policy makers. Expert Rev Neurother 16: 401-14.
 398 <http://doi.org/10.1586/14737175.2016.1155454>.
- 399 5. Feldmeier H, Heukelback J. 2009 Epidermal parasitic skin diseases: a neglected category of
 400 poverty-associated plagues. Bull World Health Organ. 87: 152-9.
 401 <http://dx.doi.org/10.2471/BLT.07.047308>.
- 402 6. Rauw W. 2012 Immune response from a resource allocation perspective. Front Genet 3: 1-14.
 403 <http://doi.org/10.3389/fgene.2012.00267>.
- 404 7. Walker PD, Flik G, Bonga SW. 2004 The biology of parasites from the genus *Argulus* and a
 405 review of the interactions with its host. In: Wiegertjes GF, Flik G, editors. Host-Parasite Interactions.
 406 New York, USA: BIOS Scientific Publishers. p. 107-29.
- 407 8. Woo PTK, Buchmann K. 2011 Fish parasites: pathobiology and protection. Cambridge: GB:
 408 MA: CABI Publishing. 362 p.
- 409 9. Giles N. 1983 Behavioural effects of the parasite *Schistocephalus solidus* (Cestoda) on an
 410 intermediate host, the three-spined stickleback, *Gasterosteus aculeatus*. Animal Behav. 31: 1192-4.
 411 [https://doi.org/10.1016/S0003-3472\(83\)80025-6](https://doi.org/10.1016/S0003-3472(83)80025-6).
- 412 10. Milinski M. 1985 Risk of predation of parasitised sticklebacks (*Gasterosteus aculeatus* L.)
 413 under competition for food. Behaviour. 93: 203-16. <https://doi.org/10.1163/156853986X00883>.
- 414 11. Giles N. 1987 Predation risk and reduced foraging activity in fish: experiments with parasitized
 415 and non-parasitized three-spined sticklebacks, *Gasterosteus aculeatus* L. J Fish Biol. 31.
 416 <https://doi.org/10.1111/j.1095-8649.1987.tb05212.x>.
- 417 12. Tierney JF, Huntingford FA, Crompton DWT. 1993 The relationship between infectivity of
 418 *Schistocephalus solidus* (Cestoda) and anti-predator behaviour of its intermediate host, the three-spined
 419 stickleback, *Gasterosteus aculeatus*. Animal Behav. 46: 603-5.
 420 <http://dx.doi.org/10.1006/anbe.1993.1229>.

- 421 13. Barber I, Huntingford FA. 1995 The effect of *Schistocephalus solidus* (Cestoda:
422 Pseudophyllidea) on the foraging and shoaling behaviour of three-spined sticklebacks, *Gasterosteus*
423 *aculeatus*. Behaviour. 132: 1223-40. <http://dx.doi.org/10.1163/156853995X00540>.
- 424 14. Barber I, Ruxton GD. 1998 Temporal prey distribution affects the competitive ability of
425 parasitized sticklebacks. Animal Behav. 56: 1477-83.
- 426 15. Ness JH, Foster SA. 1999 Parasite-associated phenotype modifications in threespine
427 stickleback. Oikos. 85. <http://dx.doi.org/10.2307/3546798>.
- 428 16. Grécias L, Valentin J, Aubin-Horth N. 2018 Testing the parasite mass burden effect on
429 alteration of host behaviour in the *Schistocephalus*-stickleback system. J Exp Biol 221: In Press.
430 <http://doi.org/10.1242/jeb.174748>.
- 431 17. Walkey M, Meakins RH. 1970 An attempt to balance the energy budget of a host-parasite
432 system. J Fish Biol. 2: 361-72. <https://doi.org/10.1111/j.1095-8649.1970.tb03294.x>.
- 433 18. Lester RJG. 1971 The influence of *Schistocephalus* plerocercoids on the respiration of
434 *Gasterosteus* and a possible resulting effect on the behavior of the fish. Can J Zool. 49: 361-6.
435 <https://doi.org/10.1139/z71-052>.
- 436 19. Barber I, Wright HA. 2005 Effects of parasites on fish behaviour: interactions with host
437 physiology. Fish Physiol. 24: 109-49. [http://dx.doi.org/10.1016/S1546-5098\(05\)24004-9](http://dx.doi.org/10.1016/S1546-5098(05)24004-9).
- 438 20. Wagner GN, McKinley RS, Bjørn PA, Finstad B. 2003 Physiological impact of sea lice on
439 swimming performance of Atlantic salmon. J Fish Biol. 62: 1000-9. [http://dx.doi.org/10.1046/j.1095-](http://dx.doi.org/10.1046/j.1095-8649.2003.00091.x)
440 [8649.2003.00091.x](http://dx.doi.org/10.1046/j.1095-8649.2003.00091.x).
- 441 21. Johnson S, Albright L. 1992 Comparative susceptibility and histopathology of the response of
442 naive Atlantic, chinook and coho salmon to experimental infection with *Lepeophtheirus salmonis*
443 (Copepoda Caligidae). Dis Aquat Organ. 14: 179-93. <http://dx.doi.org/10.3354/dao014179>.
- 444 22. Jónsdóttir H, Bron JE, Wootten R, Turnbull JF. 1992 The histopathology associated with the
445 pre-adult and adult stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. J Fish
446 Dis. 15: 521-7. <https://doi.org/10.1111/j.1365-2761.1992.tb00684.x>.
- 447 23. Östlund-Nilsson S, Curtis L, Nilsson GE, Grutter AS. 2005 Parasitic isopod *Anilocra*
448 *apogonae*, a drag for the cardinal fish *Cheilodipterus quinquelineatus*. Mar Ecol Prog Ser. 287: 209-16.
449 <https://doi.org/10.3354/meps287209>.
- 450 24. Douglas JF, Gasiorek JM, Swaffield JA, Jack L. 2011 Incompressible flow around a body.
451 Fluid Mechanics. 6th ed. Harlow, GB: Pearsons Education Limited. p. 354-82.
- 452 25. Lauder GV, Wainwright DK, Domel AG, Weaver JC, Wen L, Bertoldi K. 2016 Structure,
453 biometrics, and fluid dynamics of fish skin surfaces. Phys Rev Fluids. 1: 060502.
- 454 26. Lewis AE, Muntz WRA. 1984 The effects of external ultrasonic tagging on the swimming
455 performance of rainbow trout, *Salmo gairdneri* Richardson. J Fish Biol. 25: 577-85.
456 <https://doi.org/10.1111/j.1095-8649.1984.tb04904.x>.
- 457 27. Steinhausen MF, Andersen NG, Steffensen JF. 2006 The effect of external dummy transmitters
458 on oxygen consumption and performance of swimming Atlantic cod. J Fish Biol. 69: 951-6.
459 <https://doi.org/10.1111/j.1095-8649.2006.01143.x>.
- 460 28. Taylor NGH, Sommerville C, Wootten R. 2006 The epidemiology of *Argulus* spp. (Crustacea:
461 Branchiura) infections in stillwater trout fisheries. J Fish Dis. 29: 193-200.
462 <https://doi.org/10.1111/j.1365-2761.2006.00704.x>.
- 463 29. Menezes J, Ramos MA, Pereira TG, Moreira de Silva A. 1990 Rainbow trout culture failure in
464 a small lake as a result of massive parasitosis related to careless fish introductions. Aquaculture. 89.
465 [https://doi.org/10.1016/0044-8486\(90\)90304-6](https://doi.org/10.1016/0044-8486(90)90304-6).
- 466 30. Taylor NGH, Wootten R, Sommerville C. 2009 The influence of risk factors on the abundance,
467 egg laying habits and impact of *Argulus foliaceus* in stillwater trout fisheries. J Fish Dis. 32: 509-19.
468 <https://doi.org/10.1111/j.1365-2761.2009.01007.x>.
- 469 31. Steckler N, Yanong RPE. 2012 *Argulus* (fish louse) infections in fish. Florida: UF/IFAS
470 Fisheries and Aquatic Sciences. [Accessed:02/06/2016] Available from: <http://edis.ifas.ufl.edu/fa184>.
- 471 32. Stewart A, Jackson J, Barber I, Eizaguirre C, Paterson R, van West P, et al. 2017 Hook, line
472 and infection: a guide to culturing parasites, establishing infections and assessing immune responses in
473 the three-spined stickleback. In: Rollinson D, Stothard JR, editors. Adv Parasitol. 98: Academic Press.
474 p. 39-109. <https://doi.org/10.1016/bs.apar.2017.07.001>.

- 475 33. Stewart A, Hablützel PI, Brown M, Watson HV, Parker-Norman S, Tober AV, et al. 2018 Half
476 the story: Thermal effects on within-host infectious disease progression in a warming climate. *Glob*
477 *Chang Biol.* 24: 371-86. <https://doi.org/10.1111/gcb.13842>.
- 478 34. Stewart A, Hablützel PI, Watson HV, Brown M, Friberg IM, Cable J, et al. 2018 Physical Cues
479 Controlling Seasonal Immune Allocation in a Natural Piscine Model. *Front Immunol.* 9.
480 <http://doi.org/10.3389/fimmu.2018.00582>.
- 481 35. Schelkle B, Shinn AP, Peeler E, Cable J. 2009 Treatment of gyrodactylid infections in fish. *Dis*
482 *Aquat Organ.* 86: 65-75. <https://doi.org/10.3354/dao02087>.
- 483 36. Jayne BC, Lauder GV. 1993 Red and white muscle activity and kinematics of the escape
484 response of the bluegill sunfish during swimming. *J Comp Physiol A.* 173: 495-508.
485 <https://doi.org/10.1007/bf00193522>.
- 486 37. Domenici P, Blake R. 1997 The kinematics and performance of fish fast-start swimming. *J Exp*
487 *Biol.* 200: 1165-78.
- 488 38. Walker JA, Ghalambor CK, Griscti OL, McKenney D, Reznick DN. 2005 Do faster starts
489 increase the probability of evading predators? *Funct Ecol.* 19: 808-15. <https://doi.org/10.1111/j.1365-2435.2005.01033.x>.
- 491 39. Blake RW, Kwok PYL, Chan KHS. 2006 Effects of two parasites, *Schistocephalus solidus*
492 (Cestoda) and *Bunodera* spp. (Trematoda), on the escape fast-start performance of three-spined
493 sticklebacks. *J Fish Biol.* 69: 1345-55. <https://doi.org/10.1111/j.1095-8649.2006.01193.x>.
- 494 40. Blumstein DT, Evans CS, Daniel JC. 2007 Quantifying behaviour the JWatcher way. *Integr*
495 *Comp Biol.* 48. <https://doi.org/10.1093/icb/icn005>.
- 496 41. Gerstner CL, Webb PW. 1998 The station-holding performance of the plaice *Pleuronectes*
497 *platessa* on artificial substratum ripples. *Can J Zool.* 76: 260-8. <https://doi.org/10.1139/z97-192>.
- 498 42. Abramoff MD, Magalhaes PJ, Ram SJ. 2004 Image processing with ImageJ. *Biophotonics*
499 *International.* 11: 36-42.
- 500 43. Harper DG, Blake RW. 1990 Fast-start performance of rainbow trout *Salmo gairdneri* and
501 northern pike *Esox lucius*. *J Exp Biol.* 150: 321-42.
- 502 44. Brainerd EL, Patek SN. 1998 Vertebral column morphology, C-start curvature, and the
503 evolution of mechanical defenses in tetraodontiform fishes. *Copeia.* 971-84.
504 <http://dx.doi.org/10.2307/1447344>.
- 505 45. Bergstrom CA. 2002 Fast-start swimming performance and reduction in lateral plate number
506 in threespine stickleback. *Can J Zool.* 80: 207-13. <http://dx.doi.org/10.1139/z01-226>.
- 507 46. Brown D. 2015 Tracker Video Analysis and Modeling Tool [Accessed:02/06/2016] Available
508 from: <http://physlets.org/tracker/>.
- 509 47. R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R
510 Foundation for Statistical Computing; 2015.
- 511 48. Fox J, Weisberg S. An R companion to applied regression. Thousand Oaks, CA: Sage; 2011.
- 512 49. Venables WN, Ripley BD. 2002 Modern applied statistics with S. Fourth ed. New York:
513 Springer.
- 514 50. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4.
515 *J Stat Softw.* 67: 1-48. <http://dx.doi.org/10.18637/jss.v067.i01>.
- 516 51. Kuznetsova A, Brockhoff PB, Christensen RHB. 2017 lmerTest: tests in linear mixed effects
517 models. *J Stat Softw.* 82: 1-26. <http://dx.doi.org/10.18637/jss.v082.i13>.
- 518 52. Wickham H. 2009 ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag.
519 182 p. <http://dx.doi.org/10.1007/978-0-387-98141-3>.
- 520 53. Broell F, Burnell C, Taggart CT. 2016 Measuring abnormal movements in free-swimming fish
521 with accelerometers: implications for quantifying tag and parasite load. *J Exp Biol.* 219: 695-705.
522 <http://doi.org/10.1242/jeb.133033>.
- 523 54. Shimura S. 2009 Seasonal occurrence, sex ratio and site preference of *Argulus coregoni* Thorell
524 (Crustacea: Branchiura) parasitic on cultured freshwater salmonids in Japan. *Parasitology.* 86: 537-52.
525 <http://doi.org/10.1017/S0031182000050721>.
- 526 55. Bower-shore C. 1940 An investigation of the common fish louse, *Argulus foliaceus* (linn.).
527 *Parasitology.* 32: 361-71. <https://doi.org/10.1017/S0031182000015869>.
- 528 56. Hoffman GL. 1977 *Argulus*, a branchiuran parasite of freshwater fishes. *US Fish and Wildlife*
529 *Service.* 49: 1-9.

- 530 57. Walker P, Russon I, Haond C, Van Der Velde G, Wendelaar-Bonga S. 2011 Feeding in adult
531 *Argulus japonicus* Thiele, 1900 (maxillopoda, Branchiura), an ectoparasite on fish. *Crustaceana*. 84:
532 307-18. <http://dx.doi.org/10.1163/001121610X551881>.
- 533 58. Pottinger TG, Pickering AD, Blackstock N. 1984 Ectoparasite induced changes in epidermal
534 mucification of the brown trout, *Salmo trutta* L. *J Fish Biol*. 25: 123-8. <http://dx.doi.org/10.1111/j.1095-8649.1984.tb04857.x>.
- 535 59. Ruane NM, Nolan DT, Rotillant J, Tort L, Balm PHM, Wendelaar Bonga SE. 1999 Modulation
536 of the response of rainbow trout (*Oncorhynchus mykiss* Walbaum) to confinement, by an ectoparasitic
537 (*Argulus foliaceus* L.) infestation and cortisol feeding. *Fish Physiol Biochem*. 20: 43-51.
538 <http://dx.doi.org/10.1023/a:1007744617518>.
- 539 60. van der Salm AL, Nolan DT, Spanings FAT, Wendelaar Bonga SE. 2000 Effects of infection
540 with the ectoparasite *Argulus japonicus* (Thiele) and administration of cortisol on cellular proliferation
541 and apoptosis in the epidermis of common carp, *Cyprinus carpio* L., skin. *J Fish Dis*. 23: 173-84.
542 <http://dx.doi.org/10.1046/j.1365-2761.2000.00230.x>.
- 543 61. Barbin GP, Krueger WH. 1994 Behaviour and swimming performance of elvers of the
544 American eel, *Anguilla rostrata*, in an experimental flume. *J Fish Biol*. 45: 111-21.
545 <http://dx.doi.org/10.1111/j.1095-8649.1994.tb01290.x>.
- 546 62. Hoover JJ, Collins J, Boysen KA, Katzenmeyer AW, Killgore KJ. 2011 Critical swimming
547 speeds of adult shovelnose sturgeon in rectilinear and boundary-layer flow. *J Appl Ichthyol*. 27: 226-
548 30. <http://dx.doi.org/10.1111/j.1439-0426.2011.01707.x>.
- 549 63. Gerstner CL. 1998 Use of substratum ripples for flow refuging by Atlantic cod, *Gadus morhua*.
550 *Environ Biol Fish*. 51: 455-60. <https://doi.org/10.1023/a:1007449630601>.
- 551 64. Hockley FA, Wilson CAME, Brew A, Cable J. 2014 Fish responses to flow velocity and
552 turbulence in relation to size, sex and parasite load. *J Royal Soc Interface*. 11: 20130814.
553 <http://dx.doi.org/10.1098/rsif.2013.0814>.
- 554 65. Brassard P, Rau ME, Curtis MA. 1982 Parasite-induced susceptibility to predation in
555 diplostomiasis. *Parasitology*. 85: 495-501. <http://dx.doi.org/10.1017/S0031182000056274>.
- 556 66. Poulin R. 1994 Meta-analysis of parasite-induced behavioural changes. *Animal Behav*. 48: 137-
557 46. <http://dx.doi.org/10.1006/anbe.1994.1220>.
- 558 67. Gordon MS, Hove JR, Webb PW, Weihs D. 2000 Boxfishes as unusually well-controlled
559 autonomous underwater vehicles. *Physiol Biochem Zool*. 73: 663-71.
560 <http://dx.doi.org/10.1086/318098>.
- 561 68. Kato N. 2000 Control performance in the horizontal plane of a fish robot with mechanical
562 pectoral fins. *J Ocean Eng*. 25: 121-9. <http://dx.doi.org/10.1109/48.820744>.
- 563 69. Archer SD, Johnston IA. 1989 Kinematics of labriform and subcarangiform swimming in the
564 Antarctic fish *Notothenia neglecta*. *J Exp Biol*. 143: 195-210.
- 565 70. Walker JA, Westneat MW. 2002 Performance limits of labriform propulsion and correlates
566 with fin shape and motion. *J Exp Biol*. 205: 177-87.
- 567

568

569

570 Figure 1: Flume elevation diagram showing the flume used for the sustained swim performance tests
 571 and the characterised flow zones: Zone-1, moderately high velocity that excludes the near-bed low
 572 velocity zone; Zone-2, higher velocity downstream boundary where flow is accelerated and where fish
 573 exhausted; Zone-3, upstream near-bed boundary in which fish were observed to spend a preferential
 574 amount of time; Zone-4, low velocity near-bed boundary. Flume width is 7.6 mm. Not to scale. Vertical
 575 dotted lines indicate flow straighteners and the blue triangle indicates the water surface.

576 Figure 2: Sticklebacks were infected with *Argulus foliaceus* or sham infected a maximum of 30 min
 577 before the third flume trial (A) (indicated by red dotted line) and corresponding burst swimming trials
 578 (B) occurring 24 h later. Data are split by infection group rather than infection status; therefore, fish are
 579 only infected from Trial 3 onwards within the infected group. Sustained swimming (A), the length of
 580 time (logit transformed) that infected (n=8) and uninfected (n=5) three-spined sticklebacks
 581 (*Gasterosteus aculeatus*) were able to maintain sustained distance swimming over a series of trials as a
 582 proportion of the total time per trial (55 min). Points represent the mean and error bars are standard
 583 error extracted from a linear mixed effects model. Burst swimming (B), the velocity of infected (n=8)
 584 and uninfected (n=5) three-spined sticklebacks (*Gasterosteus aculeatus*) in the first 20 ms of a C-start
 585 escape response. Points represent the mean and error bars are standard error extracted from a linear
 586 mixed model with a square root transformation.

587 Figure 3: The proportional length of time (proportional to 55 min-logit transformed) three-spined
 588 sticklebacks (*Gasterosteus aculeatus*), uninfected (n=5) or infected (n=8) with *Argulus foliaceus* spent
 589 in (A) Zone-3 of the flume with increasing flow rate, and (B) in Zone-2, across Trials 2, 3 and 5
 590 separated by infection group (i.e. all fish are uninfected in trial 1 with the infected group being infected
 591 in the 2nd and 3rd trials). Data are extracted from LMM models, lines are the means with shaded grey
 592 95% confidence intervals (\pm CI) and points as residuals, plots are on different Y-axis scales.

593 Figure 4: The proportional length of time (logit transformed) that infected (n=8) and uninfected (n=5)
 594 three-spined sticklebacks (*Gasterosteus aculeatus*) spent holding station with increasing flow rate
 595 separated by infection status. Lines are the means with shaded grey 95% confidence intervals (\pm CI) and
 596 points as residuals.

597 Table 1: Sustained swimming performance of *Gasterosteus aculeatus* across different trials. Grey
 598 background indicates infected fish; white background is uninfected; bold text highlights significance
 599 ($p < 0.05$); analysis performed using linear mixed effects models.

600 Appendix 1: Velocity profiles (A) at different longitudinal distances along the flume (taken at the
 601 flume's centreline) measured from the upstream flow straighter (X = 0 cm) and (B) representative of
 602 each designated zone in the flume. In both graphs; Y = vertical height within flume (with Y = 0 cm the
 603 flume bed), flow rate = 1.6 L/s, blue horizontal dotted line and triangle represent the water surface,
 604 dashed lines represent means and error bars are 95% confidence intervals.

605 Appendix 2: Measured volume-averaged velocities for different flume zones. Lines represent means
 606 and error bars 95% confidence intervals (\pm CI).

607