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

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Abstract

For fish, there can be multiple consequences of parasitic infections, including the physical impacts on swimming and the pathological costs of infection. This study utilised the three-spined stickleback (*Gasterosteus aculeatus*) and the ectoparasitic fish louse, *Argulus foliaceus*, to assess both physical (including form drag and mass) and pathological effects of infection. Both sustained (prolonged swimming within an open channel flume) and burst (C-start) swimming performance were measured on individual fish before (Trials 1-2) and after infection (Trials 3-5). Experimental infection occurred shortly before the third trial, when the physical impacts of infection could be separated from any subsequent pathology as transmission of adult parasites causes instantaneous drag effects prior to observable pathology. Despite the relatively large size of the parasite and corresponding increase in hydrodynamic drag for the host, there were no observable physical effects of infection on either sustained or burst host swimming. In contrast, parasite-induced pathology is the most likely explanation for reduced swimming performance across both tests. All sticklebacks displayed a preference for flow refugia, 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    $F = \frac{1}{2} C_D \rho U_0^2 A$

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 mm^2 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 mm^2 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 ≤ 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 length^3), 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 χ^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-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-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-value=-10.417, df=226, *p*<0.001) and larger fish spent more time in Zone-2 (t-

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-

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 (χ^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 cm^2 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.

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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.

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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 (±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 (±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 (±CI).

607