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

Stewart, A[1#]., Hunt, R[1]., Mitchell, R[1]., Muhawenimana, V[2]., Wilson, C. A. M. E.[2]., Jackson, J[3]. A., Cable, J[1*].

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

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

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

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

*Corresponding Author: cablej@cardiff.ac.uk

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

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

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

Introduction

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

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

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

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

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

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

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

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

Materials and Methods

Fish and parasite origin

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

Experimental design

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

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

The Flume

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

length of the flume (Fig. 1). Swimming performance tests were conducted during daylight hours and water temperature was maintained at 22.9°C (SE±0.18) using ice blocks in the reservoir to counteract the effects of heating from the pump and the non-temperature controlled room. Haloex chloride treatment was used at 0.02 ml/L to remove chlorides and additional air bubbled into the flume reservoir using a mains operated stone aerator. A 20 mm^2 measurement grid was placed along the back sidewall of the flume to facilitate behavioural observations.

Sustained distance swim performance test

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

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

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

C-start performance test

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

Statistical analysis

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

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

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

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

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

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

Results

Impact of Argulus on host profile

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

Effect of infection on sustained and burst swimming ability

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

Fish preferences for flume zones

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

Behaviour

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

Discussion

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

In comparison to external fish tags, (26, 27) and the previous suggestions that drag from isopod infections (23) contribute to poor swimming performance, no effect of hydrodynamic drag or instability was observed in either swimming test in the current study. This is despite the parasites increasing the projected area of the fish by as much as 26.5% (mean 15.3%). For comparison, with external tagging the increase in drag force is estimated to be 12-13% for 47-72 cm cod with tags of 1.87 and 4.15 cm^2 frontal area (53). The streamlined profile of *A. foliaceus*, holding itself close to the fish's body, could explain the lack of drag and mass effects; we also checked to see if neutral buoyancy might be a possible explanation but *A. foliaceus* sink at a rate of 4.6 mm/s in a 10 ml glass measuring cylinder. It is also possible that a larger projected area increase is required to observe these effects in the laboratory, but such high intensity aggregated infections towards the head are unlikely in nature (54). Additionally, sticklebacks may be able to compensate for increased drag or instability during the early stages of infection (when only physical consequences are present), masking the physical effects of infection. The direct life cycle of *A. foliaceus* with no intermediate host means that if the host fish is consumed then the parasite's germline will also be lost, suggesting that rapid deterioration of the host is not evolutionarily favourable in this instance. A high impact on fish physiology is therefore best avoided, at least until the parasite has fed and bred.

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

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

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

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

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

Acknowledgements

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

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

References

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

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

33. Stewart A, Hablützel PI, Brown M, Watson HV, Parker-Norman S, Tober AV, et al. 2018 Half the story: Thermal effects on within-host infectious disease progression in a warming climate. Glob Chang Biol. 24: 371-86. https://doi.org/10.1111/gcb.13842.

34. Stewart A, Hablützel PI, Watson HV, Brown M, Friberg IM, Cable J, et al. 2018 Physical Cues Controlling Seasonal Immune Allocation in a Natural Piscine Model. Front Immnol. 9. http://doi.org/10.3389/fimmu.2018.00582.

35. Schelkle B, Shinn AP, Peeler E, Cable J. 2009 Treatment of gyrodactylid infections in fish. Dis Aquat Organ. 86: 65-75. https://doi.org/10.3354/dao02087.

36. Jayne BC, Lauder GV. 1993 Red and white muscle activity and kinematics of the escape response of the bluegill sunfish during swimming. J Comp Physiol A. 173: 495-508. https://doi.org/10.1007/bf00193522.

37. Domenici P, Blake R. 1997 The kinematics and performance of fish fast-start swimming. J Exp Biol. 200: 1165-78.

38. Walker JA, Ghalambor CK, Griset OL, McKenney D, Reznick DN. 2005 Do faster starts increase the probability of evading predators? Funct Ecol. 19: 808-15. https://doi.org/10.1111/j.1365-2435.2005.01033.x.

39. Blake RW, Kwok PYL, Chan KHS. 2006 Effects of two parasites, *Schistocephalus solidus* (Cestoda) and *Bunodera* spp. (Trematoda), on the escape fast-start performance of three-spined sticklebacks. J Fish Biol. 69: 1345-55. https://doi.org/10.1111/j.1095-8649.2006.01193.x.

40. Blumstein DT, Evans CS, Daniel JC. 2007 Quantifying behaviour the JWatcher way. Integr Comp Biol. 48. https://doi.org/10.1093/icb/icn005.

41. Gerstner CL, Webb PW. 1998 The station-holding performance of the plaice *Pleuronectes platessa* on artificial substratum ripples. Can J Zool. 76: 260-8. https://doi.org/10.1139/z97-192.

42. Abramoff MD, Magalhaes PJ, Ram SJ. 2004 Image processing with ImageJ. Biophotonics International. 11: 36-42.

43. Harper DG, Blake RW. 1990 Fast-start performance of rainbow trout *Salmo gairdneri* and northern pike *Esox lucius*. J Exp Biol. 150: 321-42.

44. Brainerd EL, Patek SN. 1998 Vertebral column morphology, C-start curvature, and the evolution of mechanical defenses in tetraodontiform fishes. Copeia. 971-84. http://dx.doi.org/10.2307/1447344.

45. Bergstrom CA. 2002 Fast-start swimming performance and reduction in lateral plate number in threespine stickleback. Can J Zool. 80: 207-13. http://dx.doi.org/10.1139/z01-226.

46. Brown D. 2015 Tracker Video Analysis and Modeling Tool [Accessed:02/06/2016] Available from: http://physlets.org/tracker/.

47. R Core Team. R: a lanuage and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2015.

48. Fox J, Weisberg S. An R companion to applied regression. Thousand Oaks, CA: Sage; 2011.

49. Venables WN, Ripley BD. 2002 Modern applied statistics with S. Fourth ed. New York: Springer.

50. Bates D, Mächler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. J Stat Softw. 67: 1-48. http://dx.doi.org/10.18637/jss.v067.i01.

51. Kuznetsova A, Brockhoff PB, Christensen RHB. 2017 lmerTest: tests in linear mixed effects models. J Stat Softw. 82: 1-26. http://dx.doi.org/10.18637/jss.v082.i13.

52. Wickham H. 2009 ggplot2: Elegant grpahics for data analysis. New York: Springer-Verlag. 182 p. http://dx.doi.org/10.1007/978-0-387-98141-3.

53. Broell F, Burnell C, Taggart CT. 2016 Measuring abnormal movements in free-swimming fish with accelerometers: implications for quantifying tag and parasite load. J Exp Biol. 219: 695-705. http://doi.org/10.1242/jeb.133033.

54. Shimura S. 2009 Seasonal occurrence, sex ratio and site preference of Argulus coregoni Thorell (Crustacea: Branchiura) parasitic on cultured freshwater salmonids in Japan. Parasitology. 86: 537-52. http://doi.org/10.1017/S0031182000050721.

55. Bower-shore C. 1940 An investigation of the common fish louse, *Argulus foliaceus* (linn.). Parasitology. 32: 361-71. https://doi.org/10.1017/S0031182000015869.

56. Hoffman GL. 1977 *Argulus*, a branchiuran parasite of freshwater fishes. US Fish and Wildlife Service. 49: 1-9.

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

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

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

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

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

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

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

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