

Assessing the effects of water flow rate on parasite transmission amongst a social host

Michael Reynolds  · Frances A. Hockley · Catherine A. M. E. Wilson · Jo Cable

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Abstract Many freshwater habitats are subject to change through time. Specifically, natural flow regimes are substantially modified by not only seasonal climatic change, but also anthropogenic activity. Consequently, freshwater organisms are exposed to variable flow, potentially altering their behaviour and subsequently parasite transmission amongst social hosts. Here, we investigate the effects of flow conditions on the shoaling behaviour of Trinidadian guppies (*Poecilia reticulata*), and the resulting transmission of a directly transmitted ectoparasite, *Gyrodactylus turnbulli*. Shoals exposed to continuous flow exhibited significantly greater *G. turnbulli* peak intensities and abundance when compared to fish subjected to interrupted, but not no-flow conditions. Parasite transmission rate was greater in shoals exposed to interrupted flow, resulting in parasites becoming more distributed amongst shoal members and thus reducing mean intensity in comparison to continuous flow shoals. Furthermore, as prevalence increased, the distance between shoaling

conspecifics increased at greater rates in interrupted and no-flow conditions compared to continuous flow: indicating that in the absence of flowing water, parasitism has a greater effect on shoaling decisions. This data highlights how fish behaviourally respond to variable flow conditions and the implications for parasite transmission.

Keywords Water flowrate · Shoaling · Parasite transmission · *Gyrodactylus* · *Poecilia reticulata*

Introduction

Natural variations in precipitation and the seasonal melting of mountainous snowpack can substantially modify freshwater habitats through time. Consequently, freshwater fauna are often exposed to highly variable flow conditions, which will have direct and indirect effects on fish, particularly with respect to behaviour (reviewed in Liao, 2007). Group formation of fish into loosely aggregated shoals or polarized synchronized schools, for example, provides energetic benefits for group members: the magnitude of which can depend on the water velocity to which fish are exposed (Marras et al., 2015; Ashraf et al., 2017). Whilst there are further benefits to group formation, including enhanced predator detection (Magurran, 1990) and increased foraging efficiency (Pitcher & Parrish, 1993), such behaviour can promote parasite

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M. Reynolds (✉) · F. A. Hockley · J. Cable
School of Biosciences, Cardiff University,
Cardiff CF10 3AX, UK
e-mail: reynoldsm4@cardiff.ac.uk

C. A. M. E. Wilson
School of Engineering, Cardiff University,
Cardiff CF24 3AA, UK

transmission between individuals (Barber et al., 2000). For ectoparasites with direct life cycles, the rate of direct transmission is determined by the transmission coefficient ' β ', which encompasses contact rates between infected and uninfected conspecifics, and the probability that contact results in transmission (Antonlin, 2008; McCallum et al., 2017). Historically, population-based modeling categorized transmission as either density- or frequency dependent, however, it is now recognised that both transmission modes simultaneously promote disease persistence within populations (Ryder et al., 2007; Ferrari et al., 2011; Hu et al., 2013; McCallum et al., 2017). For social species, including shoaling fish, parasite transmission between individuals may not only be affected by population density, but the frequency of contacts between infected and susceptible hosts, allowing parasite persistence even at low-host densities (Johnson & Paull, 2011).

Whilst the effects of temperature on infection risk are well documented (reviewed by Graham & Harrod, 2009; Karvonen et al., 2010), the effects associated with flow modification remain largely overlooked. Of the few studies that have investigated the relationship between flow condition and parasite transmission, the trend has been towards a higher prevalence (the number of hosts infected within a population) and intensity (the number of parasites infecting a host) within populations inhabiting reduced flows (Barker & Cone, 2000; Bodensteiner et al., 2000; Hallett & Bartholomew, 2008). However, it is difficult to disentangle whether this is because of environmental stressors (e.g., high turbidity or hypoxia) increasing the susceptibility of hosts to disease (see Leniham et al., 1999), the poor dispersal capabilities of parasites between hosts in flowing environments (Sousa & Grosholz, 1991; Hallett & Bartholomew, 2008), or if a hosts' energy resource is debilitated by infection (as in Tierney & Farrell, 2004), therefore, prompting them to frequent energetically favourable regions including shallower, slow flowing water.

Alternatively, fish can conserve energy by swimming in a conspecifics wake: a substantial benefit associated with schooling behaviour. Marras et al. (2015) showed how the energy expenditure of grey mullet swimming alone was significantly greater than those in a school, with individuals that trailed conspecifics conserving the most energy. Frequenting reduced flow velocity regions may be particularly

beneficial when shoals are exposed to variable flow conditions. However, shoaling tendencies have been observed to diminish in higher flow rates in common minnow (Garner, 1997), three-spined sticklebacks (Sneddon et al., 2006), and zebrafish (Suriyampola et al., 2017). Contrastingly, Hockley et al. (2014) showed that in no-flow conditions, the shoaling tendencies of Trinidadian guppies (*Poecilia reticulata* Peters, 1859) were significantly reduced compared to those experiencing flowing water, but only following the introduction of *Gyrodactylus turnbulli* Harris, 1986 infection. This indicated that parasitism may have a greater influence than flow in determining shoaling preferences. However, in this previous study, it was only possible to assess the immediate short-term effect of parasitism on shoaling within a 30-min time frame and not transmission dynamics over several days.

There are over 400 described *Gyrodactylus* species that are ubiquitous teleost ectoparasites infecting both tropical and temperate hosts (Bakke et al., 2007). In particular, the tropical Trinidadian guppy-*Gyrodactylus turnbulli* system has been utilised extensively to investigate social behaviour (e.g. Edenbrow et al., 2011), evolutionary ecology (Pérez-Jvostov et al., 2012; Dargent et al., 2013), and epidemiology (Stephenson et al., 2015; Smallbone et al., 2016). The parasite's direct life cycle, short generation time (ca. 24 h at 25°C; Scott, 1982) and extreme progenesis can result in exponential population growth (Bakke et al., 2007). In practical terms, parasite intensities and transmission can be accurately monitored in situ at repeated time points from the same individual, without harming or killing the host. This host-parasite system is therefore an optimal model for the current study, which aims to assess, over a prolonged period, how *G. turnbulli* transmission dynamics in shoals of Trinidadian guppies is affected by continuous, interrupted and no-flow conditions.

Materials and methods

Host and parasite origin

The experimental Trinidadian guppies (*Poecilia reticulata*) used herein were laboratory-reared descendants of a wild population caught from the Lower Aripo River, Trinidad, in 2012. Guppies were initially

maintained at Exeter University and transferred to Cardiff University in 2014. Approximately, 30 guppies were housed in each aquaria in mixed sex groups (1:5 male to female ratio), of which male fish were regularly swapped between tanks to minimise the effects of inbreeding. Despite being in captivity for 3 years prior to the experiment, and thus the magnitude of selection pressures otherwise faced in the wild having been relaxed, descendants from this laboratory population typically retain natural behaviours including predatory escape responses (Stephenson & Reynolds, 2016), social decisions (see Hockley et al., 2014; Stephenson & Reynolds, 2016) and infection dynamics (Stephenson et al., 2018). Female guppies (> 3 months) were size matched (± 3.8 mm) into groups of five individuals, representative of a natural shoal size (Croft et al., 2003). Shoals of five female guppies were deemed an appropriate number for an observer to accurately collect data during behavioural observations. Typically, 2–8 individuals have been used in previous behavioural studies (see Jones et al., 2010; Richards et al., 2010; Herbert-Rea et al. 2017). Each group was housed in 6 l aquaria to familiarize and form shoals for a minimum of 14 days (Griffiths & Magurran, 1997). Shoals were maintained under a 12 h light:12 h dark photoperiod at 22–24°C and fed daily on Aquarian® Tropical fish flakes supplemented weekly with live *Daphnia magna* Straus, 1820 and *Artemia*. For experimental infections, *Gyrodactylus turnbulli* (strain Gt3) was used: isolated from and subsequently maintained on ornamental guppies since 1997. This parasite stock is a well established, understood model that has been used to investigate host-parasite interactions for a number of years (e.g., Cable & van Oosterhout, 2007a, b; Faria et al., 2010; Schelkle et al., 2013; Mohammed et al., 2016; Stephenson et al., 2018). In the wild, it takes only one gyrodactylid to successfully infect a host, from which an epidemic within a fish population can arise (Bakke et al., 2007). Many gyrodactylids (and all *Gyrodactylus* species) demonstrate a viviparous reproductive strategy, and at least the first-born daughter is derived asexually (see Cable & Harris, 2002). Thus, in the wild, it is possible that gyrodactylid populations can be highly inbred, often descending from one individual, as was the case within our culture.

Flume set-up

Transmission experiments were conducted in three identical open-channel re-circulatory flumes, each measuring 150 cm length \times 16 cm depth \times 20 cm width (Fig. 1). Flumes were filled with dechlorinated water to a depth of 15 cm and maintained at 22–24°C. A 10 cm diameter impeller was attached to a 1hp three-phase 4-pole motor with a maximum shaft speed of 1500 rpm (Machine Mart), wired to a 1.1 kW inverter (RS Components) to control motor speed. The flume bed was set to a longitudinal gradient of 1:1000. At either end of the flume was a 2-cm thick aluminum honeycomb flow straightener with 6.4 mm cell diameter used to remove the secondary flow currents produced from the bends in the recirculatory pipe system. Flow straighteners also prevented fish from entering the impeller region, therefore, restricting shoals to a 100-cm length section (Test Arena; Fig. 1). The flow depth was fixed and maintained at 15 cm throughout all tests. Three flow conditions were tested. A static ‘no-flow’ condition (impeller off) was used as a control. A continuous ‘flow’ condition comprised a depth-averaged velocity of 6 cm s⁻¹ (measured using a Nixon miniature propeller flow meter), which corresponded to a flowrate of 1.9 l s⁻¹, and falls within the range of flow rates recorded within guppy habitats in Trinidad (Kodric-Brown & Nicoletto, 2005). A 12 h flow:12 h no-flow comprised the ‘interrupted flow’ condition (depth-averaged flow velocity 6 cm s⁻¹) and represented the time guppies frequent a shallow refuge to rest and wherein flow is minimal, particularly at night, in habitats with high-predation regimes (Seghers, 1974; Croft et al., 2003). The flow was synchronized with the rooms’ photoperiod so that fish were only exposed to flow during daylight hours.

Preliminary trial of parasite mobility

Preliminary trials assessed whether the recirculatory nature of the flumes facilitated transmission of dislodged specimens of *G. turnbulli* (hereafter ‘gyrodactylid’). Familiarised shoals of five female guppies were placed into either a continual ($n = 3$ shoals), interrupted ($n = 3$ shoals) or no-flow ($n = 3$ shoals) condition for a 24-h acclimation period, prior to gyrodactylid exposure. Following acclimation, a heavily infected donor guppy was sacrificed and

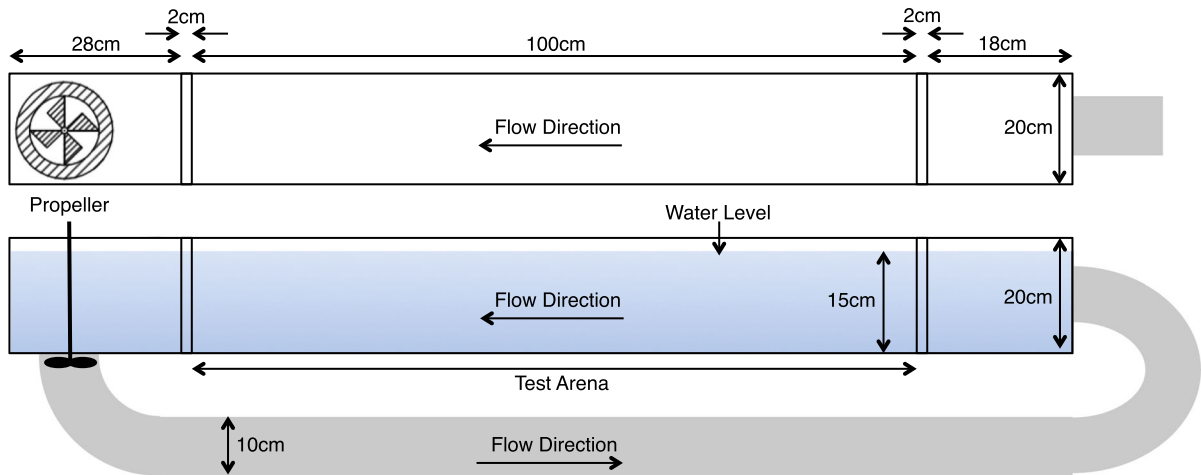


Fig. 1 2D schematic of the open-channel re-circulatory flume used to assess *Gyrodactylus turnbulli* transmission and shoaling behaviour in Trinidadian guppy shoals for three flow conditions. Top: plan view, and bottom: side view. Fish were retained

within the 100-cm-long test arena using 2-cm-thick aluminium honeycomb flow straighteners (6.4 mm cell diameter). Flow depth was maintained at 15 cm and the flume bed slope was 1 in 1000

placed in a dark cupboard for ca. 30 min, whereby such conditions encouraged worms to detach from the deceased host and attach to the water film surface (as in Cable et al., 2002). A sterile pipette was then used to transfer 30 individual worms to a watchglass containing 2 mm depth dechlorinated water, which was subsequently placed in the 18 cm terminal end of the flume (Fig. 1). After 1 h, the watchglass was removed and inspected under a dissection microscope to ensure worms had moved into the water column of the flume. The fins and skin of each guppy were screened under 0.02% tricaine methanesulfonate (MS222) sedation 24, 48 and 72 h post-gyrodactylid introduction to confirm if any worms had infected a host. If no gyrodactylids had attached to a host after 72 h (beyond *in vitro* gyrodactylid survival time; Schelkle et al., 2013), gyrodactylids were presumed dead and the trial was terminated. No ‘dislodged’ gyrodactylid reinfected a host in any treatment, confirming that any parasite transmission observed during subsequent experimental trials would be as a result of host behaviour, and not the experimental setup.

Experimental procedure

Each shoal of five female guppies ($n = 17$ shoals) was placed into a flume for a 24-h habituation period prior to a 7-day trial. A total of six shoals were trialled in the no-flow condition, five in continuous flow (one shoal

excluded due to the escape of two individuals outside of the observational arena) and six in the interrupted flow condition. On Day 0, guppies were removed from the flume and one randomly selected individual was infected with ca. 30 gyrodactylids (range 28–34 worms; no significant difference in starting intensity between treatments: ANOVA: $F_{2,16} = 1.82$, $P = 0.194$). This entailed placing a heavily infected ‘donor’ guppy within close proximity to an anaesthetised recipient guppy [using 0.02% tricaine methanesulfonate (MS222)], to allow gyrodactylids to infect the recipient through direct contact. Direct parasite transmission was observed continuously using a dissecting microscope with fibre optic illumination. The remaining four guppies were sham infected by anaesthetising and manipulating under the microscope without exposure to parasites. Each guppy was measured according to its standard length (‘SL’; mm) and profiled by making a detailed diagram of up to five unique spot patterns and/or body markings, enabling identification of individuals at repeated time points to monitor parasite transmission over the 7-day trial. On each screen day, two independent observers confirmed the identity of each individual using the detailed diagrams created at the start of a trial. Guppies were placed in 1 l-dechlorinated water for 1 h while remaining in visual contact to one another, and fed three individuals of *Daphnia magna* before being returned to the flume. Infection

was confirmed the following day (day 1), and each guppy was screened every other day thereafter (days 3, 5 and 7). For each screening, guppies were individually scooped up using a dry plastic container (in doing so preventing indirect parasite transmission via equipment) and isolated in 1 l-dechlorinated water for a maximum of 15 min, whilst remaining in visual contact. Each guppy was anaesthetised and screened to quantify prevalence (the percentage of infected individuals within the shoal), mean intensity (the mean number of parasites found on infected hosts) and abundance (the number of parasites per fish including zero counts). Transmission rate was calculated as the number of new host infections per day.

Observations of shoaling behaviour, including the number of guppies shoaling, maximum shoal size, shoal size and nearest neighbour distance (a standard measure of shoal cohesiveness), were measured at 1-min increments during a 5-min test period, every day at 9:00 am by an observer. This time was selected to coincide with peak guppy activity. Guppies were recorded as shoaling if they were within four body lengths of one another (Pitcher et al., 1983), and the number of guppies in the largest shoal defined the maximum shoal size. A 2-cm² grid adhered to the side and base of the flume was used to visually estimate the distance between neighbouring guppies whilst shoaling.

Statistical analysis

Analyses were conducted using R statistical software (version 3.1.3, R Development Core Team, 2009). Using the *lme4* library (Bates et al., 2013), Generalised linear models (GLMs) were used to investigate the effect of flow condition and fish size on *Gyrodactylus turnbulli* transmission dynamics. Dependent terms in each model included (1) *G. turnbulli* mean transmission rate, (2) *G. turnbulli* maximum prevalence, (3) time to reach maximum prevalence, (4) *G. turnbulli* maximum intensity, (5) time to reach maximum intensity, and (6) *G. turnbulli* maximum abundance. Fixed effects in these models included flow condition, shoal mean SL, and *G. turnbulli* start intensity. Models were fitted with Gaussian error families with identity or inverse link functions. Model structures were selected based on the lowest residual deviance and AIC values.

Generalised linear mixed-models were constructed to assess the influence of parasitism, fish size and flow condition on the shoaling behaviour of Trinidadian guppies. Dependent terms within these ‘global’ models included (6) the number of guppies shoaling, and (7) the mean nearest neighbour distances between shoaling conspecifics. The independent terms included flow condition (no-flow, interrupted or continual flow), *G. turnbulli* mean intensity and prevalence (as defined by Bush et al., 1997), host mean SL, day and *G. turnbulli* start intensity. Interaction terms between flow × parasite mean intensity, flow × prevalence and flow × day were also incorporated into the models. To account for repeated measures, shoal number was included as a random term. Models were fitted with Gaussian error structure and log link functions, and model robustness assessed using residual plots.

During model refinement, several equally well-supported models were identified based on comparisons of Akaike’s Information Criterion (AIC). Thus, an information theoretic approach to multi-model inference was employed to assess the relative importance of each independent term in influencing dependent variables within the global GLMMs (following methods in Burnham & Anderson, 2002). As variables were measured on different scales, model parameters within each global model (containing all independent, dependent and random terms) were standardized to a mean of 0 and standard deviation 0.5 using the *arm* library (Grueber et al., 2011; Gelman & Su, 2013). The ‘dredge’ function within the *MuMIn* package (Barton, 2013) was then used to generate a set of ‘top’ models, which fell within 2.5 AICc of the best model. Averaged parameter estimates from this top set of models were then calculated using the ‘model.avg’ function, and the relative importance of each parameter generated by summing the Akaike weights across the models in which the parameter occurred (Burnham & Anderson, 2002).

Results

Shoals exposed to interrupted flow exhibited significantly greater gyrodactylid mean transmission rates compared to those in continuous and no-flow conditions (Table 1, Model 1; Fig. 2a). Over time, prevalence increased in all treatments, and a greater mean

Table 1 Results output from general linear models investigating the effects of flow condition, fish size and *Gyrodactylus turnbulli* starting intensity on *G. turnbulli* transmission dynamics in Trinidadian guppy shoals

Model	Dependent variable	Predictor	Estimate	SE	T value	P value
1	Mean transmission rate	Intercept	1.053	0.516	2.041	0.064
		Flow condition				
		Continuous flow	0.176	0.080	2.187	0.049*
		Interrupted	0.241	0.068	3.549	0.004**
		Mean SL	-0.010	0.009	-1.059	0.310
		Start intensity	-0.018	0.016	-1.105	0.291
2	Maximum prevalence	Intercept	188.531	46.631	4.043	0.002
		Flow condition				
		Continuous flow	17.945	7.255	2.473	0.004**
		Interrupted	22.159	6.132	3.614	0.029*
		Mean SL	-0.785	0.811	-0.968	0.352
		Start intensity	-3.186	1.475	-2.160	0.052
3	Time to maximum prevalence	Intercept	6.861	4.861	1.411	0.184
		Flow condition				
		Interrupted	-0.630	0.639	-0.985	0.344
		Continuous flow	-0.112	0.756	-0.148	0.885
		Mean SL	0.036	0.085	0.431	0.674
		Start intensity	-0.027	0.154	-0.177	0.863
4	Maximum intensity	Intercept	0.093	0.012	7.673	<0.001
		Flow condition				
		Interrupted	0.008	0.004	2.290	0.041*
		Continuous flow	-0.002	0.003	-0.771	0.456
		Mean SL	-0.001	0.0002	-2.429	0.032*
		Start intensity	-0.002	0.0004	-5.123	<0.001***
5	Time to maximum intensity	Intercept	14.942	10.695	1.397	0.188
		Flow condition				
		Interrupted	-2.425	1.406	-1.724	0.110
		Continuous flow	-0.488	1.664	-0.293	0.775
		Mean SL	-0.358	0.186	-1.928	0.078
		Start intensity	-0.095	0.338	-0.281	0.783
6	Maximum abundance	Intercept flow condition	67.788	38.091	1.780	0.121
		Interrupted	-1.064	5.256	-0.202	0.843
		Continuous flow	18.159	6.216	2.921	0.014*
		Mean SL	-1.811	0.737	-2.457	0.030*
		Start intensity	0.068	1.340	0.051	0.960

The level of significance is denoted by an asterisk as follows: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$

transmission rate in interrupted conditions resulted in a significantly higher mean peak prevalence of 100% being reached, compared to 92% and 83% in continuous and no-flow conditions, respectively (Table 1, Model 2).

Significantly higher gyrodactylid mean intensities and abundance were reached in continuous flow compared to interrupted, but not no-flow conditions (Table 1, Models 4 and 6; Fig. 2b). Shoals that had a

higher gyrodactylid start intensity also reached higher intensities (Table 1, Model 4). Additionally, there was a positive correlation between the mean SL of a shoal and gyrodactylid mean intensity (Table 1, Model 4). There was no effect of flow condition, a shoals mean standard length or gyrodactylid start intensity on the time taken to reach maximum gyrodactylid prevalence or intensity during the experimental period (Table 1, Models 3 and 5, respectively).

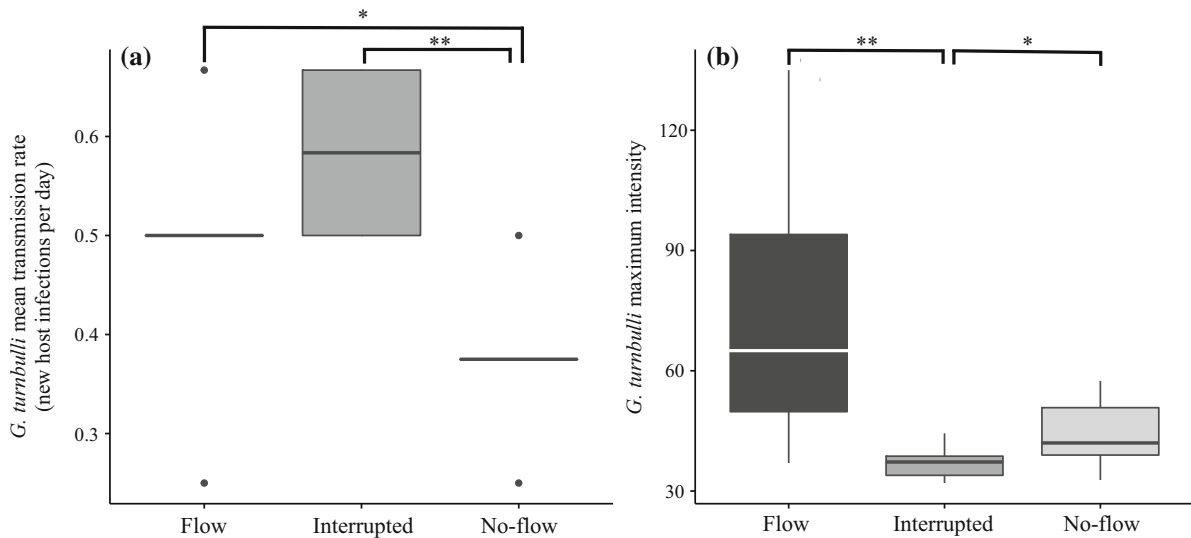


Fig. 2 Mean *Gyrodactylus turnbulli* transmission rate (a), and *G. turnbulli* mean intensity (b) within shoals exposed to continuous flow (black), interrupted (dark grey), and no-flow (light grey) conditions. Black dots represent outliers; bars the

infection with gyrodactylids had a significant effect on the shoaling behaviour of guppies. As parasite prevalence and intensity increased, the number of guppies shoaling decreased: significantly more so within flowing, compared to interrupted or no-flow conditions (Table 2, Model 6). As parasite prevalence increased, the distances between shoaling conspecifics increased at greater rates in interrupted and no-flow treatments compared to continuous flow conditions (Fig. 3); indicating that in the absence of flowing water, parasitism may have a greater effect on shoaling behaviour (Prevalence \times flow interaction: Table 2, Model 7).

Discussion

The current study investigated *Gyrodactylus turnbulli* transmission dynamics within a social host, the Trinidadian guppy, under prolonged exposure to continuous, interrupted and no-flow conditions. A significantly greater gyrodactylid transmission rate was observed in interrupted flow, resulting in higher parasite prevalence compared to continuous and no-flow conditions. As gyrodactylids reproduce in situ (Cable & Harris, 2002), the transmission potential amongst hosts increases as infrapopulation densities

also increase (Bakke et al., 2007). Competition for resources and evasion of a hosts' immune response (see Rubio-Godoy et al., 2012) prompt parasite transmission to new hosts, resulting in the parasite population becoming more evenly distributed throughout a shoal. Within interrupted flow conditions, there may be multiple transmission opportunities. First, through direct contacts as hosts school in flowing water, and, second, during a nocturnal 'rest' period when the impeller was switched off. Although guppies are presumed, a diurnal species with shoaling tendencies ceasing as light intensity diminishes (O'Connor & Krause, 2003), nocturnal behaviours, for example prolonged foraging activity, have been observed particularly in the absence of predation (see Fraser et al., 2004). Furthermore, *G. turnbulli*-infected individuals exhibit nocturnal restlessness (Reynolds & Cable, submitted): frequently encountering and initiating body contact with resting conspecifics during which parasite transmission may occur (Croft et al. 2011). Similarly, for shoals in no-flow conditions, transmission may predominantly occur via nocturnal activity of infected fish moving between resting conspecifics attempting to 'offload' their parasite burdens (Reynolds et al., 2017). The success of which, however, may also depend on the behaviour of the 'recipient'. For example, guppies can discriminate

Table 2 Summary of the standardized averaged model predictors explaining variation in shoaling behaviour of *Gyrodactylus turnbulli* infected Trinidadian guppies within continuous, interrupted and no-flow conditions

Model	Dependent variable	Predictor	Estimate	Adjusted SE	95% CI		Relative importance of predictor
					2.5%	97.5%	
6	Number of fish shoaling	Intercept	1.189	0.067	1.058	1.320	
		Day	-0.206	0.101	-0.404	-0.008	0.48
		Flow condition					0.39
		Continuous flow	-0.235	0.101	-0.437	-0.033	
		Interrupted	-0.022	0.087	-0.196	0.151	
		Mean Intensity	-0.103	0.051	-0.203	-0.003	0.47
		Mean SL	0.192	0.094	0.009	0.376	0.73
		Prevalence	-0.237	0.089	-0.411	-0.063	0.71
		Start intensity	0.069	0.088	-0.104	0.241	0.14
7	Nearest neighbour distance	Intercept	0.414	0.033	0.348	0.480	
		Day	0.151	0.044	0.065	0.237	1.00
		Day × flow condition					0.17
		Day × continuous flow	-0.104	0.066	-0.234	0.026	
		Day × interrupted	0.014	0.067	-0.118	0.145	
		Flow condition					1.00
		Continuous flow	0.151	0.049	0.056	0.247	
		Interrupted	0.024	0.047	-0.069	0.118	
		Mean intensity	0.001	0.034	-0.066	0.068	0.09
		Mean SL	0.012	0.040	-0.067	0.091	0.09
		Prevalence	0.020	0.092	-0.161	0.201	0.22
		Prevalence × flow condition					0.12
		Prevalence × continuous flow	-0.131	0.079	-0.286	0.023*	
		Prevalence × interrupted	0.012	0.078	-0.139	0.164	
Start Intensity	-0.037	0.041	-0.118	0.044	0.13		

Highlighted rows show predictors that confidence intervals did not bound zero and are therefore considered significant
CI confidence intervals, *SE* standard error, *SL* Standard length

*Approaching significance

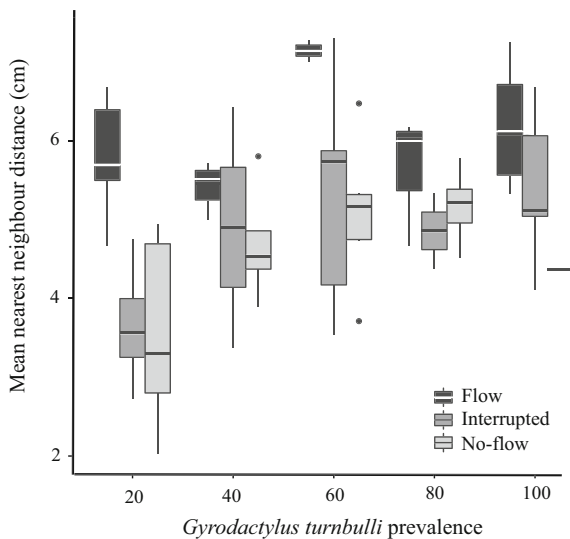


Fig. 3 The nearest neighbour distances between shoaling conspecifics in relation to *Gyrodactylus turnbulli* prevalence in continuous (black, $n = 5$), interrupted (dark grey, $n = 6$), and no-flow (light grey, $n = 6$) conditions. Black dots represent outliers; bars the upper and lower limits; the box the first and third quartile with median

between *G. turnbulli*-infected and uninfected conspecifics using olfactory cues alone, and respond by initiating evasive behaviours towards infected individuals (Stephenson & Reynolds, 2016). The perception of cues indicative of infection may be enhanced in static compared to flowing environments, prompting fish to avoid infected conspecifics and thus reducing parasite transmission opportunities. This may support why shoals in no-flow conditions had reduced prevalence, compared to those in interrupted and continuous flow conditions.

Maximum gyrodactylid intensity and abundance observed during the study period was significantly greater within continually flowing environments than both interrupted and no-flow conditions and thus a greater transmission rate may have been expected. However, parasites infecting these fish were subject to continuous water flow over the hosts' body, and although not measured here, coupled with an increased tail beat frequency may have been more vulnerable to dislodgement. Scott & Anderson (1984) estimated that ca. 40% of *G. turnbulli* became dislodged whilst attempting transmission from donor to recipient fish in static water alone, which is likely to be significantly greater within flowing conditions. Following dislodgement, mortality is inevitable should a parasite fail to

encounter a new host, as indicated within our preliminary trials.

A reduced prevalence within continuous flow shoals, compared to interrupted flow shoals, would have resulted in greater mean parasite intensities, whereby a smaller proportion of hosts were infected with the majority of parasites. Additionally, whilst guppies exhibit both innate and acquired immunity towards gyrodactylid infections (Scott, 1985; Cable & van Oosterhout, 2007a), the efficiency of these responses may become compromised if energy reserves are diverted to alternative fitness-related traits, including locomotion (as in Zamora-Camacho et al., 2015; Husak et al., 2016). We speculate that guppies enduring continual flow may divert energy resources into locomotion and consequently compromise immunity to infection, compared to conspecifics experiencing no-flow conditions. This would allow parasite proliferation, particularly on larger hosts which provide more space and resources for parasites. Here, greater parasite intensities were reached in shoals of larger guppies, which is consistent with observations in wild fish, which generally harbour more parasites with maximum parasite loads increasing exponentially with host body size (see Cable & van Oosterhout, 2007b).

As *G. turnbulli* prevalence and intensity increased, the number of fish shoaling decreased across all treatments, significantly more so within continuous flow conditions. Additionally, we confirm the findings of Richards et al. (2010) that the distances between shoaling guppies increased with parasite prevalence. Shoals became less cohesive at a greater rate within interrupted and no-flow conditions, indicating that in the absence of flowing water, parasitism may have a greater effect on shoaling behaviour. A reduction in shoaling tendency associated with parasitism has been reported in several previous studies (Dugatkin et al., 1994; Krause & Godin, 1996; Hockley et al., 2014), which is not surprising given it is beneficial for individuals to discriminate between infected and uninfected conspecifics, and respond accordingly (see Stephenson & Reynolds, 2016). By remaining within a shoal, but increasing nearest neighbour distances, an individual can simultaneously experience the benefits of swimming in the reduced velocity region of a conspecific's wake (Marras et al., 2015), whilst also reducing infection risk. When the costs associated with *G. turnbulli* infection (reviewed in

Bakke et al., 2007) outweigh the benefits of remaining with a group, individuals may isolate themselves reducing the overall number of fish shoaling, as observed here. Such isolative sickness behaviour is not uncommon and is demonstrated across multiple taxa (insects; Rueppell et al., 2010, birds; Brown & Brown, 1992, mammals; Proudfoot et al., 2014). Furthermore, exclusion of infected individuals by uninfected conspecifics, and/or the failure of an infected fish in sustaining its shoaling position, particularly within flowing environments (see van Oosterhout et al., 2007), may contribute to a reduction in shoaling tendency.

Conclusions

The specific goal of this study was to identify how variable flow conditions impact parasite transmission dynamics and shoaling behaviour in a social host. Our results highlight how flow alteration substantially modifies parasite transmission dynamics, whereby parasitism was more prevalent in shoals exposed to interrupted flow conditions due to greater transmission rates. Additionally, fish experiencing continuous flow developed intense parasite infections: likely having severe implications for host health. In no-flow conditions, parasitism also appeared to have a greater significance on host shoaling behaviour. It is important to note that only female guppies were used during this experiment due to their greater propensity to shoal than males (Croft et al., 2003). In the wild, male guppies move between female shoals in search of mating opportunities, which could subsequently enhance parasite transmission between individuals (see Richards et al., 2010, 2012). Nevertheless, an understanding of how disease ecology is affected by river flow variability is particularly important considering that parasite infections can substantially modify host population dynamics. Future works should consider extending experimental durations so as to identify peaks in infection parameters and their associated costs for host health and behaviour at a population level.

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Author contributions FH and JC designed the study; CAMEW provided technical advice; MR and FH collected the data; MR performed statistical analysis and drafted the manuscript; all authors commented on manuscript drafts.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed. Procedures and protocols were conducted under UK Home Office license (PPL 303424) with approval by the Cardiff University Animal Ethics Committee.

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