

RESEARCH ARTICLE

Transmission risk predicts avoidance of infected conspecifics in Trinidadian guppies

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Funding information

The Fisheries Society of the British Isles; Center for Adaptation to a Changing Environment (ACE); ETH Zürich

Handling Editor: Niels Dingemans

Abstract

1. Associating with conspecifics afflicted with infectious diseases increases the risk of becoming infected, but engaging in avoidance behaviour incurs the cost of lost social benefits. Across systems, infected individuals vary in the transmission risk they pose, so natural selection should favour risk-sensitive avoidance behaviour that optimally balances the costs and benefits of sociality.
2. Here, we use the guppy *Poecilia reticulata*–*Gyrodactylus turnbulli* host–parasite system to test the prediction that individuals avoid infected conspecifics in proportion to the transmission risk they pose.
3. In dichotomous choice tests, uninfected fish avoided both the chemical and visual cues, presented separately, of infected conspecifics only in the later stages of infection.
4. A transmission experiment indicated that this avoidance behaviour accurately tracked transmission risk (quantified as both the speed at which transmission occurs and the number of parasites transmitting) through the course of infection.
5. Together, these findings reveal that uninfected hosts can use redundant cues across sensory systems to inform dynamic risk-sensitive avoidance behaviour. This correlation between the transmission risk posed by infected individuals and the avoidance response they elicit has implications for the evolutionary ecology of infectious disease, and its explicit inclusion may improve the ability of epidemic models to predict disease spread.

KEYWORDS

effective contact rate (β), group living, infectious disease avoidance behaviour, parasite transmission, redundant multimodal cues, risk-sensitive behaviour, social behaviour, social evolution

1 | INTRODUCTION

Social interactions between individuals influence infectious disease dynamics at the population level (Aiello et al., 2016; Clay, Lehmer, Previtali, St. Jeor, & Dearing, 2009; Gear, Perkins, & Hudson, 2009), so understanding factors affecting these interactions and how they change in the presence of disease will facilitate more accurate predictions of how diseases spread (Aiello et al., 2016; Hawley, Etienne, Ezenwa, & Jolles, 2011; Lloyd-Smith, Schreiber, Kopp, & Getz, 2005;

Paul et al., 2012; VanderWaal & Ezenwa, 2016). Social animals associating with infected conspecifics likely increase their risk of infection, particularly with directly transmitted disease-causing organisms, and there is evidence from multiple taxa that they avoid doing so (Behringer, Butler, & Shields, 2006; Croft et al., 2011; Goodall, 1986; Kavaliers, Fudge, Colwell, & Choleris, 2003; Kiesecker, Skelly, Beard, & Preisser, 1999; Poirotte et al., 2017; Schaller, 2011). For many animals, such “social barriers” to disease transmission may be as important as immunological or physical ones (Loehle, 1995; Schaller, 2011;

Zylberberg, Klasing, & Hahn, 2013). However, engaging in avoidance behaviour incurs the cost of lost social benefits (e.g. antipredator defence, foraging efficiency and mating opportunities: Croft et al., 2011; Seppälä, Karvonen, & Valtonen, 2008; Schaller, 2011).

The outcome of this trade-off may be determined by the probability contact with a particular infected individual will result in transmission or its “infectiousness.” Infectiousness is highly heterogeneous in natural populations: The vast majority of transmission events involve a minority of infected individuals (Lloyd-Smith et al., 2005; Paull et al., 2012). How infectious an individual is depends on the characteristics of its infection. For example, across a variety of systems, the number of parasites an individual is infected with, its “infection load,” is an important predictor of the number of infectious particles it releases and hence the transmission risk it poses to uninfected conspecifics (e.g. Aiello et al., 2016; Matthews et al., 2006; Stephenson et al., 2017). As well as variation between individuals, a single individual's infection load and hence infectiousness is, for many disease systems, likely to change through the course of infection (Poulin, 2007; Schmid-Hempel, 2011). Infection duration also encompasses variation in the strength of the host's immune response, symptoms and behaviour, as well as the demography of the infecting parasites and their ability to transmit and establish infections on new hosts (Aiello et al., 2016; Bakke, Cable, & Harris, 2007; Charleston et al., 2011; Chase-Topping, Gally, Low, Matthews, & Woolhouse, 2008; Fraser et al., 2014; Schmid-Hempel, Pühr, Krüger, Reber, & Schmid-Hempel, 1999; Scott & Anderson, 1984; Therese & Bashey, 2012). Given this heterogeneity, natural selection should favour the evolution of mechanisms that maximize the cost–benefit balance of association and avoidance, such as avoidance behaviour that is sensitive to the transmission risk posed by individual conspecifics.

The prediction that uninfected individuals mitigate the risk posed by infectious individuals by modulating their own avoidance behaviour can be formalized using an epidemiological modelling framework. In such models, the effective contact rate, β , is the product of the contact rate between infected and uninfected individuals (behavioural component of β , β_c) and the transmission rate per contact, which is often driven by the infected hosts' response to the parasites, mediated by infection load (physiological component of β , β_p ; Anderson & May, 1991; Lloyd-Smith et al., 2005; Hawley et al., 2011; VanderWaal & Ezenwa, 2016). Historically, models have assumed homogeneous population mixing and transmission risk, that is mean field estimates of β_c and β_p , but this typically leads to overestimated transmission rates (Keeling & Grenfell, 2000). More recent work has demonstrated that incorporating empirical estimates of heterogeneity in both β_c and β_p improves model fit to natural disease dynamics (see Aiello et al., 2016 and references therein), but that β_c and β_p may themselves covary has been largely ignored. However, this covariation has potentially powerful implications for disease dynamics. For example, using a simple modelling framework, Hawley et al. (2011) showed that behaviourally mediated covariation in β_c and β_p , such as risk-sensitive avoidance of infectious conspecifics, can mean the difference between a parasite invading a host population or fading

out. Despite this, empirical tests of how β_c and β_p covary in natural systems are still lacking (Hawley et al., 2011; VanderWaal & Ezenwa, 2016).

We used the guppy *Poecilia reticulata*–*Gyrodactylus turnbulli* host–parasite system to experimentally test for risk-sensitive avoidance of infectious conspecifics. *G. turnbulli* is an ectoparasitic monogenean that reproduces on the host's skin with a generation time of 24 hr and transmits directly through close contact between socially interacting hosts (Stephenson, van Oosterhout, Mohammed, & Cable, 2015). *Gyrodactylus* spp. parasites are the most prevalent multicellular parasites in wild guppy populations (Stephenson, van Oosterhout, Mohammed, et al. 2015) and are associated with reduced guppy body condition (Stephenson, van Oosterhout, & Cable, 2015), attractiveness (Kennedy, Endler, Poynton, & McMinn, 1987) and survival (Stephenson, Kinsella, Cable, & van Oosterhout, 2016; van Oosterhout et al., 2007). The ability to recognize and avoid infected individuals is therefore likely to be under strong selection and there is some evidence that it occurs; the presence of infected conspecifics reduces shoal cohesion in semi-natural conditions (Croft et al., 2011). However, the loss of shoal cohesion as a result of this infection-avoidance behaviour carries a cost: Less cohesive fish shoals are more vulnerable to predation (Seppälä et al., 2008). If guppies balance this trade-off by employing risk-sensitive avoidance of infected conspecifics, avoidance should be positively correlated with infection duration: Infection load initially increases over the course of infection and is an important predictor of transmission risk (Stephenson et al., 2017).

Beyond favouring the evolution of risk-sensitive behaviour, natural selection should favour the use of cues appropriate to the sensory environment. For example, in static water bodies, chemical cues may provide reliable information, but turbidity may limit the usefulness of visual cues; correspondingly, tadpoles use chemical but not visual cues to avoid infected conspecifics (Kiesecker et al., 1999). By contrast, in habitats characterized by dynamic sensory environments, selection should favour the use of multiple sensory modalities to detect and respond to redundant cues (i.e. those that elicit the same response in receivers when presented in isolation; Partan & Marler, 2005). Such cue redundancy is most likely to evolve in habitats in which no single sense is continuously informative. Rivers, such as those inhabited by guppies, experience turbulent flow and turbidity; as a result, visual and chemical cues elicit redundant risk-sensitive antipredator behaviour in several riverine fishes (e.g. the naked characin, *Gymnocharacinus bergi*; see Cordi, Ortubay, & Lozada, 2005). Guppies may use similarly redundant visual and chemical cues in risk-sensitive infection-avoidance behaviour. Previous work has shown that they are able to use chemical cues to monitor temporally variable physiological characteristics in conspecifics (reproductive status: Brask, Croft, Thompson, Dablesteen, & Darden, 2012; disease: Stephenson & Reynolds, 2016) and have excellent vision (Anstis, Hutahajan, & Cavanagh, 1998). However, visual cues of infection may provide a general “sickness” cue and include behaviour, which host animals are able to modify in the short term to conceal their disease (e.g. Lopes, Adelman, Wingfield, &

Bentley, 2012). Chemical cues potentially provide more honest, less easily manipulable information about health, which may also be specific to the disease-causing agent: Guppies may therefore respond differently to cues across these sensory modalities.

We here test the prediction that social hosts display risk-sensitive avoidance of infected conspecifics that pose the highest risk of transmission. We presented uninfected “test” guppies with a dichotomous choice between the cues (visual or chemical, presented separately) of *G. turnbulli*-infected and uninfected conspecific “stimulus” fish. Uninfected guppies avoided both chemical and visual cues of infected conspecifics only in the later stages of infection. Models developed from a transmission experiment using this system (Stephenson et al., 2017) predicted that both transmission speed and the number of parasites transmitting increase through the course of the infection on the stimulus fish. Indeed, days on which the predicted risk was highest were those on which avoidance was strongest. These results comprise the first demonstration that infection-avoidance behaviour is sensitive to present infection risk (β_c and β_p are negatively correlated) and therefore highlight a potentially important and under-studied source of variation in infectious disease transmission.

2 | MATERIALS AND METHODS

2.1 | Host and parasite origin and maintenance

We used wild-caught guppies and their laboratory-bred descendants from the Caura River, Trinidad, and a single strain of the parasite *Gyrodactylus turnbulli* (Gt3). Guppies were housed at low densities in 70 L aquaria at $24 \pm 1^\circ\text{C}$, on a 12-hr light: 12-hr dark lighting schedule (overhead fluorescent lighting), and fed daily on Aquarian® flakes, supplemented with *Artemia* and bloodworm. Gt3 was originally isolated from an ornamental guppy and has been maintained on inbred ornamental stocks (“culture fish”) in the laboratory since 1997.

2.2 | Chemical and visual cue production

We used F1 laboratory-bred virgin females to produce the chemical and visual cues of infection. These “stimulus pairs” (uninfected vs. infected, $n = 28$ pairs) were size-matched ± 1 mm. Recently killed infected Gt3 culture fish were placed in close proximity to the anaesthetized (0.02% tricaine methanesulphonate; MS222; PHARMAQ Ltd., Fordingbridge, UK) stimulus fish until two parasites had transferred, as observed under a dissecting microscope and fibre optic illumination. The stimulus fish were revived and housed individually in 1-L tanks, and the number of parasites infecting each was counted under anaesthetic every other day. As a handling control, uninfected stimulus fish were also anaesthetized and held individually in 1-L tanks. All tanks were maintained under standard conditions and received 100% water exchanges every other day. We exclusively used female guppies as stimulus fish because male guppies typically have complex and highly polymorphic colour patterns that affect how

both male and female conspecifics respond to them (reviewed in e.g. Houde, 1997). By only using females, therefore, we avoided the substantial challenge of standardizing male colour patterns among and between pairs.

The pairs of infected and uninfected fish were used to produce chemical stimuli for the behavioural trials. Due to a change in experimental design, chemical cues were produced either in batches or pairs. During the production of each batch, five fish were held individually, each in 500 ml of dechlorinated water in food grade plastic containers for 24 hr. Fish were not fed during this isolation. These 500 ml fish conditioned water samples were then mixed and frozen in 150 ml aliquots at -20°C . During the production of paired chemical cues, the same protocol was followed except that the samples from each stimulus fish were kept separate (see Supporting Information Appendix S1: Table S1 for more details).

2.3 | Avoidance behaviour experiment

We exposed uninfected guppies (“test fish”) to the chemical ($n = 87$) and visual ($n = 83$) cues of the stimulus pairs. All test and stimulus fish were unfamiliar to one another, that is they had never been in the same or adjacent stock tanks. We manipulated the length of time the infected stimulus fish had been infected and measured the avoidance behaviour elicited in the test fish. We used a 30×60 cm tank, filled to 5 cm water depth (Supporting Information Appendix S1: Figure S1). At one end of the tank, we placed two glass cylinders with adjacent Nalgene® tubing, separated by an opaque barrier. At the other end was a settling compartment (10×30 cm), separated from the test arena by a removable opaque barrier. For the chemical cue trials, cues were introduced via the Nalgene® tubing at 10 ml/min, maintained by flow metres (MMA-35, Dwyer Instruments, High Wycombe, UK). Test fish of both sexes were taken from the wild-caught parental and F2 generations (see Supporting Information Appendix S1: Table S1) and tested individually. Fish acclimatized in the settling compartment for 10 min. For the visual cue trials, stimulus pairs were placed in the glass cylinders, one fish per cylinder, before this acclimatization period. The glass cylinders were entirely watertight and washed inside and out between trials with 70% ethanol and clean water: no chemical cues of the stimulus pair could have been detected by the test fish during the visual cue trials. In chemical trials, the flow of chemical cues (infected vs. uninfected) was started two min before the end of acclimatization. The barrier was lifted remotely via a pulley system at the end of the acclimatization period, and a 10-min test period began when the fish crossed into the test arena. After each trial, the tank and components were rinsed with 70% ethanol and clean water. The sex of the test fish and the side of the tank that received the cue of infected conspecific were changed between trials according to a Latin square design. All behavioural trials were video-recorded for later analysis using JWATCHER™ 1.0 (www.jwatcher.ucla.edu).

We used different measures of association for the two senses to accommodate inherent differences between them: chemical cues could be detected across the whole side of the tank, while visually

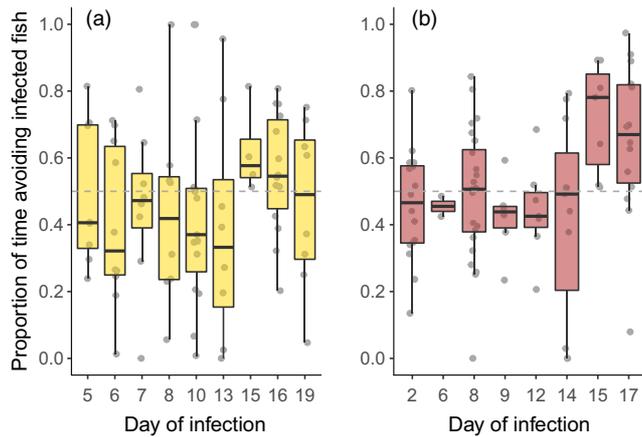


FIGURE 1 Uninfected guppies avoided *Gyrodactylus turnbulli*-infected conspecifics only when these were in the later stages of infection, based on both visual (a) and chemical (b) cues. The points give the raw data, thick lines the median, boxes the first and third quartiles, and whiskers extend to the largest and smallest value within 1.5× the interquartile range

mediated preference is typically measured in time spent in proximity to the stimulus fish (Houde, 1997). For chemical cue trials, therefore, we used the proportion of the 10-min test period that test fish spent on the side of the tank that received the cue of the uninfected fish. For visual cue trials, we used the proportion of time test fish spent on the side of the “end zone” next to the uninfected fish out of the total time (out of the 10-min test period) that test fish spent in the end zone (Supporting Information Appendix 1: Figure S1).

2.4 | Predicting transmission risk

To predict the transmission risk posed by the infected stimulus fish on each day of infection on which they were used as stimuli, we used models built on data from a transmission experiment using this system (for detailed methods and results see Stephenson et al., 2017). In brief, we experimentally infected parasite-naïve laboratory-bred females descended from guppies caught in the lower Aripo River, Trinidad (“donors,” $n = 60$), using the methods and Gt3 parasite strain described above. We exclusively used female fish in this experiment to minimize variation in transmission attributable to the differences in behaviour between male and female guppies. We housed the donors individually in 1-L tanks and allowed them to develop natural variation in infection loads. On Days 5 and 12 of infection, parasite-naïve female “recipients” were size-matched to the donors ± 2 mm and added to the tanks. The number of *G. turnbulli* parasites on both donor and recipient was recorded daily. Once transmission had occurred, the recipient was removed from the tank. We thus observed 105 transmission events and used the data to construct generalized linear mixed models (GLMMs) explaining variation in how quickly transmission occurred (“transmission speed”) and how many parasites transmitted (“transmission load”). The best-supported model for transmission speed included only the donor’s infection load at

the time of transmission and that for transmission load included donor infection load, donor infection integral (i.e. the area under the curve of its infection load over time) and the day of infection of the donor (Stephenson et al., 2017). Using these models and the infection load, infection integral and day of infection on which they were used, we obtained the model predictions of the transmission speed and load of the stimulus fish in the behavioural experiment. To accommodate the substantial uncertainty around these predicted values, we simulated the predictions 1,000 times for both models (Nicolaus et al., 2012).

2.5 | Data analysis

We analysed the data using R 3.3.1 (R Core Team 2016) and provide the data, script and output in Supporting Information Appendix S1. We used the proportion of time the test fish spent associated with the uninfected stimulus fish cue (i.e. avoiding the infected stimulus fish cue) as the response variable in a GLMM (beta error distribution with logit link function in the glmmADMB package; Fournier et al., 2012). As fixed effects, we included the day of infection and infection integral (i.e. the area under the curve of its infection load over time) of the stimulus fish; test fish sex and standard length; the cue type used (chemical or visual); and the side of the tank in which the cue of infected conspecific was placed (to test for any side bias). We also included the year in which the tests were conducted, which encompassed changes in test fish generation (wild-caught parental vs. laboratory-bred F2) and in stimulus production method (batch vs. pair; see Supporting Information Appendix S1: Table S1 for more details). We included the two-way interactions between test fish sex, cue type (visual or chemical), day of infection and infection integral about which we had a priori hypotheses. The identity of the stimulus pair used in a trial was included as a random term as each was used on multiple days. The full output of this model is presented in Supporting Information Appendix S1.

We used GLMMs to test whether the predicted transmission speed and transmission load of the stimulus fish increased through time. Both for models testing transmission speed and transmission load, we included day of infection as a fixed effect and the stimulus pair identity as a random effect to control for the fact that each was used on multiple days (Gamma error family, log link function in lme4; Bates, Maechler, Bolker, & Walker, 2015). We applied these models to each simulation of the values predicted by the transmission models, resulting in 1,000 estimates each of how transmission speed and transmission load change through the course of infection on the stimulus fish. We here present the mean and bootstrapped 95% confidence intervals of these values. Where the confidence intervals do not bound 0, transmission risk changes significantly through time.

3 | RESULTS

The full output and model fits for all models are given in Supporting Information Appendix S1. The length of time the stimulus fish had

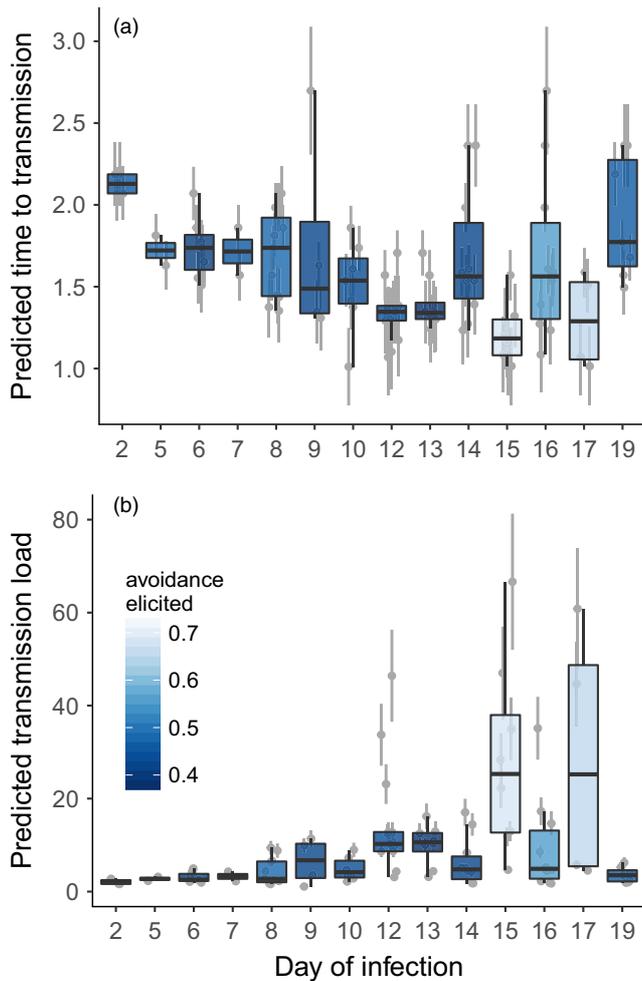


FIGURE 2 The predicted speed (in days) at which transmission would occur (a), and the number of parasites transmitting (b) from the stimulus fish increased through the course of infection and covaried with the avoidance behaviour they elicited. The points give the values (mean of 1,000 simulations \pm 1 standard error) predicted by models built on data from 105 transmission events (from the experiment presented in Stephenson et al., 2017) and using the infection load, infection integral (i.e. the area under the curve of its infection load over time) and day of infection of the stimulus fish in the present experiment. Thick lines denote the median values, boxes the first and third quartiles and whiskers extend to the largest and smallest value within $1.5\times$ the interquartile range. The shading of the boxes denotes the mean behavioural avoidance elicited by the stimulus fish on each day of infection, as given by the scale bar (raw data in Figure 1). One outlying data point (with a mean predicted transmission load of 90) has been omitted from (b) for clarity

been infected (day of infection) was the only variable that explained variation in the proportion of time test fish spent avoiding the infected stimulus fish, with test fish only avoiding stimulus fish in the later stages of infection ($\chi^2 = 9.84$, $p = 0.0017$; Figure 1). There was no significant effect of cue type, or its interaction with day of infection, indicating redundancy between the visual and chemical cues. The predicted transmission speed (mean estimate over 1,000

simulations = -0.007 ; bootstrapped 95% CI = -0.008 to -0.006) and transmission load (mean estimate over 1,000 simulations = 0.073 ; bootstrapped 95% CI = 0.072 – 0.075) of the stimulus fish increased through the course of their infection (Figure 2).

In post hoc tests investigating the apparent threshold at Day 15 of infection, we found no difference between test fish response to chemical and visual cues (main effect) or how visually and chemically mediated behaviour changed depending on the duration of the infection of the stimulus fish (pre vs. postday 15 interaction with cue type), again indicating redundancy between these multimodal cues. Guppies marginally but significantly preferred (i.e. spent more than 50% of the time associating with) conspecifics infected for fewer than 15 days over uninfected counterparts ($M \pm SE = 0.55 \pm 0.02$, $t_{122} = 2.56$, $p = 0.012$), but strongly avoided those infected for longer than 15 days (i.e. spent $<50\%$ of the time with; $M \pm SE = 0.40 \pm 0.03$, $t_{46} = -3.16$, $p = 0.0027$). Pre- and post-15 day stimulus fish elicited significantly different responses in test fish ($\chi^2 = 15.15$, $p < 0.0001$). Moreover, postday 15 infection stimulus fish had significantly higher predicted transmission loads (mean estimate over 1,000 simulations = 0.32 ; bootstrapped 95% CI = 0.30 – 0.34), but not speeds (mean estimate over 1,000 simulations = 0.002 ; bootstrapped 95% CI = -0.007 to 0.01), than preday 15 stimulus fish.

4 | DISCUSSION

We tested whether natural selection has driven the evolution of infection-avoidance behaviour that could potentially optimally balance the costs and benefits of sociality. In a dichotomous choice test, uninfected guppies avoided both the visual and chemical cues, presented separately, of *Gyrodactylus turnbulli*-infected conspecifics only in the later stages of infection (Figure 1). Predictions of the transmission risk posed by these infected conspecifics from models built on data from a transmission experiment using this system (Stephenson et al., 2017) illustrated that this avoidance behaviour tracked transmission risk through time, such that those that posed the highest predicted risk were most strongly avoided (Figure 2). Our data represent unique empirical evidence that the two components of the effective contact rate β (contact rate, β_c , and infectiousness, β_p) covary quantitatively, rather than as a binary comparison of infected and uninfected individuals.

Both chemical and visual cues for avoidance behaviour may be primarily derived from the host and its response to the parasite, rather than from the parasite itself. This suggestion is based on two observations. First, stimulus fish infection duration, rather than infection load, was the most important predictor of avoidance behaviour in this study. Second, guppies that have imprinted on the chemical cues of conspecifics experiencing *G. turnbulli*-induced disease, but that have been parasite-free for over a month, preferentially associate with the chemical cues of conspecifics in the late stages of *G. turnbulli* infection (Stephenson & Reynolds, 2016). There thus appears to be a host-derived chemical cue of *G. turnbulli*-induced disease that elicits behavioural responses in conspecifics.

Parasite-derived cues may not elicit a response because directly transmitted parasites are under strong selection to conceal their presence on the host, thereby increasing their chances of transmitting to new hosts (Poulin, 2007). Indeed, malaria parasites strategically control the emission of chemical cues to maximize their fitness, attracting vectors particularly strongly when they are ready to transmit (Cornet, Nicot, Rivero, & Gandon, 2013; De Moraes et al., 2014).

Infectious hosts should also be under strong selection to disguise their infection in order to continue benefitting from group living and to increase their relative fitness by transmitting parasites to unrelated group mates. In other systems, hosts conceal pathology and sickness behaviour (Lopes et al., 2012), and early in infection, the guppies in our experiment also appear to do so successfully and are even marginally more attractive than their uninfected counterparts. This counterintuitive observation may be due to the infected stimulus fish interacting more with the test fish or having a generally higher activity level than the uninfected fish; infected fish tend to initiate more social interactions in semi-natural conditions (Croft et al., 2011).

The many potential cues of infection likely become increasingly difficult to suppress through the course of infection: In our data, a critical threshold in cue composition or concentration appears to be reached after 15 days of infection. One component may be alarm cue, a chemical released from fish skin damaged during predation events and infection (Poulin, Marcogliese, & McLaughlin, 1999), which elicits avoidance behaviour in guppies and many other species (Brown, Macnaughton, Elvidge, Ramnarine, & Godin, 2009 and references therein). Other chemical cues may be related to epithelial cell composition or mucous chemistry, both of which change during the course of gyrodactylid infection (Buchmann & Lindenstrøm, 2002; Gheorghiu, Marcogliese, & Scott, 2012). The parasite itself may use chemical cues from the host, or conspecifics, to determine when the benefits of transmission outweigh the risks (Stephenson, 2012; Stephenson et al., 2017): Such cues may therefore accurately reflect the real-time probability of parasite transmission. The visual cues of infection also become more obvious as the infection progresses. For example, guppies may display clamped fins, paleness and difficulty swimming (Kennedy et al., 1987). In addition, during later stages of infection, gyrodactylid-infected guppies attempt to “rub up” against shoalmates (Croft et al., 2011). This abnormal behaviour itself, and the opportunity it provides shoalmates to sample the host’s chemical and visual cues at close range, potentially explains their observed avoidance by conspecifics in semi-natural conditions (Croft et al., 2011). Indeed, it is likely to be the abnormality of these cues, rather than what they signify, that guppies avoid (Stephenson & Reynolds, 2016).

If the cues of infection are indeed host-derived and independent of infection load, as our data suggest, the infection-avoidance behaviour they mediate could be widespread in natural populations despite the relatively low infection loads observed in field surveys (Stephenson, van Oosterhout, Mohammed, et al., 2015). Further, while the cues in our experiment were presented separately, in natural settings, guppies are likely often in receipt of both. Together, they

could have an effect equal to that of either cue alone or the response could be greater (Partan & Marler, 2005); guppies are more attentive to visual cues when in receipt of chemical cues (Stephenson, 2016). In avoiding infected individuals, guppies in natural populations also benefit from avoiding predators that might use the same cues to find relatively easy prey (Stephenson et al., 2016). Indeed, ostracizing infected individuals, thereby facilitating their capture by predators, may have the added benefit of reducing population-level parasite prevalence and intensity (Packer, Holt, Hudson, Lafferty, & Dobson, 2003) and thus the per capita infection risk. In a further contrast with the natural setting, we constrained the stimulus fish in this experiment, but previous work on this and other systems suggests that infection may increase or decrease their attempts to interact (Croft et al., 2011; Lopes, Block, & König, 2016). Future work should elucidate how the behaviour of infected and uninfected hosts interacts with the infectiousness of infected hosts in driving disease transmission.

Our results highlight the importance of accounting for the feedback between host and parasite during the infection process in modelling the spread of infectious diseases (Ezenwa et al., 2016): a particular pitfall if basing such inference on empirically derived static social networks of uninfected animals (e.g. references in Rushmore, Bisanzio, & Gillespie, 2017). Modelling approaches provide one solution to this issue by incorporating the uncertainty associated with the codynamics of network structure and infection into static models, offering insight where the interplay is an empirical unknown (Silk et al., 2017). However, we have shown that disease can have a quantitative, nonlinear effect on the contact behaviour of social animals, indicating that using dynamic models explicitly incorporating this feedback between infection and behaviour will likely improve predictions (Farine, 2017). The relationship between β_c and β_p may also drive evolutionary change in both host and parasite. For example, heritable variation between uninfected hosts in their ability to avoid infected conspecifics (Zylberberg et al., 2013), and between infected hosts in their ability to transmit the parasite (Boots, White, Best, & Bowers, 2012), can shape the evolution of host defence mechanisms. In addition, disease transmission and the interactions between infected and susceptible hosts drive the evolution of parasite virulence (e.g. Lion & Boots, 2010). In the light of its potentially profound importance for the evolutionary ecology of disease, further empirical and theoretical consideration of the relationship between β_c and β_p and the factors affecting it are sorely needed.

ACKNOWLEDGEMENTS

This work was conducted under the UK Home Office licence (PPL 302876) with approval by the Cardiff University Animal Ethics Committee. J. Fox, A. Hunt, R. S. Mohammed and M. Reynolds provided technical assistance. A. Felmy, J. Jokela, R. J. Thomas and K. A. Young made helpful comments on earlier versions of this manuscript. The Fisheries Society of the British Isles (FSBI; PhD studentship to J.F.S.) and the Center for Adaptation to a Changing

Environment (ACE) at ETH Zürich (fellowship to J.F.S.) funded this work.

AUTHORS' CONTRIBUTIONS

J.F.S. conceived the study, designed and conducted the behavioural experiment, analysed all data, interpreted the results, wrote and revised the manuscript. S.E.P. and J.C. designed and conducted the transmission experiment. All authors gave final approval for publication and agree to be accountable for the accuracy and integrity of the work.

DATA ACCESSIBILITY

Data supporting the results are archived in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.c1090r5> (Stephenson, Perkins, & Cable, 2018).

REFERENCES

- Aiello, C. M., Nussear, K. E., Esque, T. C., Emblidge, P. G., Sah, P., Bansal, S., & Hudson, P. J. (2016). Host contact and shedding patterns clarify variation in pathogen exposure and transmission in threatened tortoise *Gopherus agassizii*: Implications for disease modelling and management. *Journal of Animal Ecology*, *85*, 829–842. <https://doi.org/10.1111/1365-2656.12511>
- Anderson, R. M., & May, R. M. (1991) *Infectious diseases of humans*. Oxford, UK: Oxford University Press.
- Anstis, S., Hutahajan, P., & Cavanagh, P. (1998). Optomotor test for wavelength sensitivity in guppyfish (*Poecilia reticulata*). *Vision Research*, *38*, 45–53. [https://doi.org/10.1016/S0042-6989\(97\)00159-4](https://doi.org/10.1016/S0042-6989(97)00159-4)
- Bakke, T. A., Cable, J., & Harris, P. D. (2007). The biology of gyrodactylid monogeneans: The “Russian-doll killers”. *Advances in Parasitology*, *64*, 161–460. [https://doi.org/10.1016/S0065-308X\(06\)64003-7](https://doi.org/10.1016/S0065-308X(06)64003-7)
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). *lme4: Linear mixed-effects models using Eigen and S4*.
- Behringer, D. C., Butler, M. J. D., & Shields, J. (2006). Avoidance of disease by social lobsters. *Nature*, *441*, 421. <https://doi.org/10.1038/441421a>
- Boots, M., White, A., Best, A., & Bowers, R. (2012). The importance of who infects whom: The evolution of diversity in host resistance to infectious disease. *Ecology Letters*, *15*, 1104–1111. <https://doi.org/10.1111/j.1461-0248.2012.01832.x>
- Brask, J. B., Croft, D. P., Thompson, K., Dablesteen, T., & Darden, S. K. (2012). Social preferences based on sexual attractiveness: A female strategy to reduce male sexual attention. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *279*, 1748–1753. <https://doi.org/10.1098/rspb.2011.2212>
- Brown, G. E., Macnaughton, C. J., Elvidge, C. K., Ramnarine, I. W., & Godin, J.-G. J. (2009). Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian guppies. *Behavioral Ecology and Sociobiology*, *63*, 699–706. <https://doi.org/10.1007/s00265-008-0703-4>
- Buchmann, K., & Lindenstrøm, T. (2002). Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology*, *32*, 309–319. [https://doi.org/10.1016/S0020-7519\(01\)00332-0](https://doi.org/10.1016/S0020-7519(01)00332-0)
- Charleston, B., Bankowski, B. M., Gubbins, S., Chase-Topping, M., Schley, D., Howey, R., ... Woolhouse, M. E. (2011). Relationship between clinical signs and transmission of an infectious disease and the implications for control. *Science*, *332*, 726–729. <https://doi.org/10.1126/science.1199884>
- Chase-Topping, M., Gally, D., Low, C., Matthews, L., & Woolhouse, M. E. (2008). Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nature Reviews: Microbiology*, *6*, 904–912. <https://doi.org/10.1038/nrmicro2029>
- Clay, C. A., Lehmer, E. M., Previtali, A., St. Jeor, S., & Dearing, M. D. (2009). Contact heterogeneity in deer mice: Implications for Sin Nombre virus transmission. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *276*, 1305–1312. <https://doi.org/10.1098/rspb.2008.1693>
- Cordi, V., Ortubay, S., & Lozada, M. (2005). Visual cues during the alarm reaction of *Gymnocharacinus bergi* (Pisces, Characidae). *Journal of Applied Ichthyology*, *21*, 487–491. <https://doi.org/10.1111/j.1439-0426.2005.00660.x>
- Cornet, S., Nicot, A., Rivero, A., & Gandon, S. (2013). Malaria infection increases bird attractiveness to uninfected mosquitoes. *Ecology Letters*, *16*, 323–329. <https://doi.org/10.1111/ele.12041>
- Croft, D. P., Edenbrow, M., Darden, S. K., Ramnarine, I. W., van Oosterhout, C., & Cable, J. (2011). Effect of gyrodactylid ectoparasites on host behaviour and social network structure in guppies, *Poecilia reticulata*. *Behavioral Ecology and Sociobiology*, *65*, 2219–2227. <https://doi.org/10.1007/s00265-011-1230-2>
- De Moraes, C. M., Stanczyk, N. M., Betz, H. S., Pulido, H., Sim, D. G., Read, A. F., & Mescher, M. C. (2014). Malaria-induced changes in host odors enhance mosquito attraction. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 11079–11084. <https://doi.org/10.1073/pnas.1405617111>
- Ezenwa, V. O., Archie, E. A., Craft, M. E., Hawley, D. M., Martin, L. B., Moore, J., & White, L. (2016). Host behaviour-parasite feedback: An essential link between animal behaviour and disease ecology. *Proceedings of the Royal Society of London B: Biological Sciences*, *283*, 20153078. <https://doi.org/10.1098/rspb.2015.3078>
- Farine, D. (2017). The dynamics of transmission and the dynamics of networks. *Journal of Animal Ecology*, *86*, 415–418. <https://doi.org/10.1111/1365-2656.12659>
- Fournier, D. A., Skaug, H. J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M., ... Sibert, J. (2012). AD model builder: Using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software*, *27*, 233–249. <https://doi.org/10.1080/10556788.2011.597854>
- Fraser, C., Lythgoe, K., Leventhal, G. E., Shirreff, G., Hollingsworth, T. D., Alizon, S., & Bonhoeffer, S. (2014). Virulence and pathogenesis of HIV-1 infection: An evolutionary perspective. *Science*, *343*, 1243727. <https://doi.org/10.1126/science.1243727>
- Gheorghiu, C., Marcogliese, D. J., & Scott, M. E. (2012). Waterborne zinc alters temporal dynamics of guppy *Poecilia reticulata* epidermal response to *Gyrodactylus turnbulli* (Monogenea). *Diseases of Aquatic Organisms*, *98*, 143–153. <https://doi.org/10.3354/dao02434>
- Goodall, J. (1986). Social rejection, exclusion, and shunning among the Gombe chimpanzees. *Ethology and Sociobiology*, *7*, 227–236. [https://doi.org/10.1016/0162-3095\(86\)90050-6](https://doi.org/10.1016/0162-3095(86)90050-6)
- Gear, D. A., Perkins, S. E., & Hudson, P. J. (2009). Does elevated testosterone result in increased exposure and transmission of parasites? *Ecology Letters*, *12*, 528–537. <https://doi.org/10.1111/j.1461-0248.2009.01306.x>
- Hawley, D. M., Etienne, R. S., Ezenwa, V. O., & Jolles, A. E. (2011). Does animal behavior underlie covariation between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease dynamics. *Integrative and Comparative Biology*, *51*, 528–539. <https://doi.org/10.1093/icb/1093/062>
- Houde, A. (1997). *Sex, color and mate choice in guppies*. Princeton, NJ: Princeton University Press.
- Kavaliers, M., Fudge, M. A., Colwell, D. D., & Choleris, E. (2003). Aversive and avoidance responses of female mice to the odors of

- males infected with an ectoparasite and the effects of prior familiarity. *Behavioral Ecology and Sociobiology*, 54, 423–430. <https://doi.org/10.1007/s00265-003-0631-2>
- Keeling, M. J., & Grenfell, B. T. (2000). Individual-based perspectives on R_0 . *Journal of Theoretical Biology*, 203, 51–61. <https://doi.org/10.1006/jtbi.1999.1064>
- Kennedy, C. E. J., Endler, J. A., Poynton, S. L., & McMinn, H. (1987). Parasite load predicts mate choice in guppies. *Behavioral Ecology and Sociobiology*, 21, 291–295. <https://doi.org/10.1007/BF00299966>
- Kiesecker, J. M., Skelly, D. K., Beard, K. H., & Preisser, E. (1999). Behavioral reduction of infection risk. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 9165–9168. <https://doi.org/10.1073/pnas.96.16.9165>
- Lion, S., & Boots, M. (2010). Are parasites “prudent” in space? *Ecology Letters*, 13, 1245–1255. <https://doi.org/10.1111/j.1461-0248.2010.01516.x>
- Lloyd-Smith, J. O., Schreiber, S. J., Kopp, P. E., & Getz, W. M. (2005). Superspreading and the effect of individual variation on disease emergence. *Nature*, 438, 355–359. <https://doi.org/10.1038/nature04153>
- Loehle, C. (1995). Social barriers to pathogen transmission in wild animal populations. *Ecology*, 76, 326–335. <https://doi.org/10.2307/1941192>
- Lopes, P. C., Adelman, J., Wingfield, J. C., & Bentley, G. E. (2012). Social context modulates sickness behaviour. *Behavioral Ecology and Sociobiology*, 66, 1421–1428. <https://doi.org/10.1007/s00265-012-1397-1>
- Lopes, P. C., Block, P., & König, B. (2016). Infection-induced behavioural changes reduce connectivity and the potential for disease spread in wild mice contact networks. *Scientific Reports*, 6, 31790. <https://doi.org/10.1038/srep31790>
- Matthews, L., Low, J. C., Gally, D. L., Pearce, M. C., Mellor, D. J., Heesterbeek, J. A. P., ... Woolhouse, M. E. (2006). Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 547–552. <https://doi.org/10.1073/pnas.0503776103>
- Nicolaus, M., Tinbergen, J. M., Bouwman, K. M., Michler, S. P. M., Ubels, R., Both, C., ... Dingemans, N. J. (2012). Experimental evidence for adaptive personalities in a wild passerine bird. *Proceedings of the Royal Society of London B: Biological Sciences*, 279, 4885–4892. <https://doi.org/10.1098/rspb.2012.1936>
- Packer, C., Holt, R. D., Hudson, P. J., Lafferty, K. D., & Dobson, A. P. (2003). Keeping the herds healthy and alert: Implications of predator control for infectious disease. *Ecology Letters*, 6, 797–802. <https://doi.org/10.1046/j.1461-0248.2003.00500.x>
- Partan, S. R., & Marler, P. (2005). Issues in the classification of multimodal communication signals. *The American Naturalist*, 166, 231–245. <https://doi.org/10.1086/431246>
- Paull, S. H., Song, S., McClure, K. M., Sackett, L. C., Kilpatrick, A. M., & Johnson, P. T. J. (2012). From superspreaders to disease hotspots: Linking transmission across hosts and space. *Frontiers in Ecology and the Environment*, 10, 75–82. <https://doi.org/10.1890/110111>
- Poirotte, C., Massot, F., Herbert, A., Willaume, E., Bomo, P. M., Kappeler, P. M., & Charpentier, M. J. E. (2017). Mandrills use olfaction to socially avoid parasitized conspecifics. *Science Advances*, 3, e1601721. <https://doi.org/10.1126/sciadv.1601721>
- Poulin, R. (2007). *Evolutionary ecology of parasites*, 2nd ed. Princeton, NJ: Princeton University Press.
- Poulin, R., Marcogliese, D. J., & McLaughlin, J. D. (1999). Skin-penetrating parasites and the release of alarm substances in juvenile rainbow trout. *Journal of Fish Biology*, 55, 47–53. <https://doi.org/10.1111/j.1095-8649.1999.tb00655.x>
- R Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rushmore, J., Bisanzio, D., & Gillespie, T. R. (2017). Making new connections: Insights from primate-parasite networks. *Trends in Parasitology*, 33, 547–560. <https://doi.org/10.1016/j.pt.2017.01.013>
- Schaller, M. (2011). The behavioural immune system and the psychology of human sociality. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, 366, 3418–3426. <https://doi.org/10.1098/rstb.2011.0029>
- Schmid-Hempel, P. (2011). *Evolutionary parasitology*. Oxford, UK: Oxford University Press.
- Schmid-Hempel, P., Pühr, K., Krüger, N., Reber, C., & Schmid-Hempel, R. (1999). Dynamic and genetic consequences of variation in horizontal transmission for a microparasitic infection. *Evolution*, 53, 426–434. <https://doi.org/10.2307/2640779>
- Scott, M. E., & Anderson, R. M. (1984). The population dynamics of *Gyrodactylus bullatarudis* (Monogenea) within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology*, 89, 159–194. <https://doi.org/10.1017/S0031182000001207>
- Seppälä, O., Karvonen, A., & Valtonen, E. T. (2008). Shoaling behaviour of fish under parasitism and predation risk. *Animal Behaviour*, 75, 145–150. <https://doi.org/10.1016/j.anbehav.2007.04.022>
- Silk, M. J., Croft, D. P., Delahay, R. J., Hodgson, D. J., Weber, N., Boots, M., & McDonald, R. A. (2017). The application of statistical network models in disease research. *Methods in Ecology and Evolution*, 8, 1026–1041. <https://doi.org/10.1111/2041-210X.12770>
- Stephenson, J. F. (2012). The chemical cues of male sea lice *Lepeophtheirus salmonis* encourage others to move between host Atlantic salmon *Salmo salar*. *Journal of Fish Biology*, 81, 1118–1123. <https://doi.org/10.1111/j.1095-8649.2012.03347.x>
- Stephenson, J. F. (2016). Keeping eyes peeled: Guppies exposed to chemical alarm cue are more responsive to ambiguous visual cues. *Behavioral Ecology and Sociobiology*, 70, 575–584. <https://doi.org/10.1007/s00265-016-2076-4>
- Stephenson, J. F., Kinsella, C., Cable, J., & van Oosterhout, C. (2016). A further cost for the sicker sex? Evidence for male-biased parasite-induced vulnerability to predation. *Ecology and Evolution*, 6, 2506–2515. <https://doi.org/10.1002/ece3.2049>
- Stephenson, J. F., Perkins, S. E., & Cable, J. (2018). Data from: Transmission risk predicts avoidance of infected conspecifics in Trinidadian guppies. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.c1090r5>
- Stephenson, J. F., & Reynolds, M. (2016). Imprinting can cause a maladaptive preference for infectious conspecifics. *Biology Letters*, 12, 20160020. <https://doi.org/10.1016/j.cub.2010.08.013>
- Stephenson, J. F., van Oosterhout, C., & Cable, J. (2015). Pace of life, predators and parasites: Predator-induced life history evolution in Trinidadian guppies predicts decrease in parasite tolerance. *Biology Letters*, 11, 20150806. <https://doi.org/10.1098/rsbl.2015.0806>
- Stephenson, J. F., van Oosterhout, C., Mohammed, R. S., & Cable, J. (2015). Parasites of Trinidadian guppies: Evidence for sex- and age-specific trait-mediated indirect effects of predators. *Ecology*, 96, 489–498. <https://doi.org/10.1890/14-0495.1>
- Stephenson, J. F., Young, K. A., Fox, J., Jokela, J., Cable, J., & Perkins, S. E. (2017). Host heterogeneity affects both parasite transmission to and fitness on subsequent hosts. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, 372, 20160093. <https://doi.org/10.1098/rstb.2016.0093>
- Therese, M. O., & Bashey, F. (2012). Natal-host environmental effects on juvenile size, transmission success, and operational sex ratio in the entomopathogenic nematode, *Steinernema carpocapsae*. *Journal of Parasitology*, 98, 1095–1100. <https://doi.org/10.1645/GE-3069.1>
- van Oosterhout, C., Mohammed, R. S., Hansen, H., Archard, G. A., McMullan, M., Weese, D. J., & Cable, J. (2007). Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *International Journal for Parasitology*, 37, 805–812. <https://doi.org/10.1016/j.ijpara.2006.12.016>
- VanderWaal, K. L., & Ezenwa, V. (2016). Heterogeneity in pathogen transmission: Mechanisms and methodology. *Functional Ecology*, 30, 1606–1622. <https://doi.org/10.1111/1365-2435.12645>
- Zylberberg, M., Klasing, K. C., & Hahn, T. P. (2013). House finches (*Carpodacus mexicanus*) balance investment in behavioural and

immunological defences against pathogens. *Biology Letters*, 9, 20120856. <https://doi.org/10.1098/rsbl.2012.0856>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Stephenson JF, Perkins SE, Cable J. Transmission risk predicts avoidance of infected conspecifics in Trinidadian guppies. *J Anim Ecol.* 2018;00:1–9. <https://doi.org/10.1111/1365-2656.12885>