

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <https://orca.cardiff.ac.uk/id/eprint/105100/>

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

Citation for final published version:

Reynolds, Michael , Arapi, Elissavet and Cable, Joanne 2018. Parasite-mediated host behavioural modifications: *Gyrodactylus turnbulli* infected Trinidadian guppies increase contact rates with uninfected conspecifics. *Parasitology* 145 (7) , pp. 920-926. 10.1017/S0031182017001950

Publishers page: <https://doi.org/10.1017/S0031182017001950>

Please note:

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

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



1 **Title:** Parasite-mediated host behavioural modifications: *Gyrodactylus turnbulli* infected
2 Trinidadian guppies increase contact rates with uninfected conspecifics

3

4 **Running title:** Adaptive guppy responses to *Gyrodactylus* infection

5

6 Michael Reynolds*, Elisavet A. Arapi, Jo Cable

7

8 School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK

9 Corresponding author*

10 reynoldsm4@cardiff.ac.uk

11 [+44\(0\)7477165806](tel:+44(0)7477165806)

12

13

14

15

16

17

18

19

20

21

22

23

24

25 **SUMMARY**

26

27 While group formation provides antipredatory defences, increases foraging efficiency and
28 mating opportunities, it can be counterintuitive by promoting disease transmission amongst
29 social hosts. Upon introduction of a pathogen, uninfected individuals often modify their
30 social preferences to reduce infection risk. Infected hosts also exhibit behavioural changes,
31 for example, removing themselves from a group to prevent an epidemic. Conversely, here we
32 show how Trinidadian guppies infected with a directly transmitted ectoparasite, *Gyrodactylus*
33 *turnbulli*, significantly increase their contact rates with uninfected conspecifics. As
34 uninfected fish never perform this behaviour, this is suggestive of a parasite-mediated
35 behavioural response of infected hosts, presumably to offload their parasites. In the early
36 stages of infection, however, such behavioural modifications are ineffective in alleviating
37 parasite burdens. Additionally, we show that fish exposed to *G. turnbulli* infections for a
38 second time, spent less time associating than those exposed to parasites for the first time. We
39 speculate that individuals build and retain an infection cue repertoire, enabling them to
40 rapidly recognise and avoid infectious conspecifics. This study highlights the importance of
41 considering host behavioural modifications when investigating disease transmission
42 dynamics.

43

44 **Key words:** *Gyrodactylus*; *Poecilia reticulata*; sociality; infectious disease; transmission
45 dynamics; behavioural modification

46

47

48

49

50 KEY FINDINGS

- 51 • *Gyrodactylus turnbulli* infected guppies significantly increase contact rates with
52 conspecifics.
- 53 • This adaptive behavioural response of the host is presumably aimed at ‘offloading’
54 parasites.
- 55 • In the early stages of infection, such behaviour is not an effective strategy for reducing
56 parasite burdens.
- 57 • Fish exposed to secondary infections associated for less time than those experiencing
58 primary infections.
- 59 • This study highlights the importance of considering adaptive host behavioural changes
60 when investigating disease dynamics.

61

62 INTRODUCTION

63

64 Sociality confers many benefits, particularly with respect to antipredatory defences (Krause
65 and Ruxton, 2002; Sansom *et al.* 2009). Group formation can, however, be counterintuitive
66 by increasing competition for scarce resources, mating opportunities, and the
67 conspicuousness of prey to predators, all of which can directly impede fitness (Alexander,
68 1974; Sherman *et al.* 1995). Most notable is increased disease susceptibility owing to chronic
69 stress induced by social competition (Glaser and Kiecolt-Glaser, 2005; Proudfoot and
70 Habing, 2015; Schneider *et al.* 2016), and the close proximity of conspecifics facilitating
71 disease propagation amongst hosts (Côté and Poulin, 1995). This often results in more
72 intense, prevalent infections as group size increases (reviewed in Patterson and Ruckstuhl,
73 2013).

74

75 At a population level, a significant determinant of a disease transmission pathway is a
76 group's social structure, which arises from consistent interaction patterns between
77 conspecifics (Whitehead, 1997; Wey *et al.* 2008). The rate of disease transmission depends
78 on the 'socialness' and infection status of group members. Well-connected individuals, for
79 example, are at greater risk of acquiring infections (Bell *et al.* 1999; Danon *et al.* 2011), and
80 can subsequently act as superspreaders of disease (Lloyd-Smith *et al.* 2005; Craft, 2015). As
81 infection becomes more prevalent within a population, conspecifics often respond to visual
82 and/or chemical cues indicative of infection by modifying their associative preferences to
83 reduce their infection risk. Such behavioural avoidance has been documented in a number of
84 taxa including amphibians (Kiesecker *et al.* 1999; Koprivnikar and Penalva, 2015), fish
85 (Ward *et al.* 2005) and mammals (Curtis, 2014; Poirotte *et al.* 2017).

86

87 Infected hosts often show remarkable adaptive behavioural traits aimed at combatting
88 disease. Infected fish, for example, frequent warmer thermal conditions outside the thermal
89 tolerance of their associated pathogens to 'self-medicate' against infection (see Mohammed
90 *et al.* 2016). Alternatively, infected hosts can isolate themselves to protect the integrity of a
91 group. Extreme forms of altruistic behaviour, such as 'altruistic suicide', have been observed
92 within social insects including ants and honeybees; upon acquiring infection, these hosts
93 remove themselves from a colony to prevent disease epidemics (Heinze and Walter, 2010;
94 Rueppell *et al.* 2010). Conversely, by forming larger groups, parasite 'attacks' can become
95 diluted amongst group members (Duncan and Vigne, 1979; Rätti *et al.* 2006), with such
96 encounter-dilution effects mitigating parasite-mediated costs of sociality (Mooring and Hart,
97 1992).

98

99 Whether or not infected individuals increase their contact rates with uninfected conspecifics
100 remains questionable. Indeed, ‘offloading’ parasites onto conspecifics could be beneficial in
101 terms of alleviating parasite burdens and their associative costs, whilst also serving to
102 ‘vaccinate’ recipients against subsequent infections (Faria *et al.* 2010). Using the well-studied
103 social Trinidadian guppy and its directly transmitted ectoparasite, *Gyrodactylus turnbulli*, we
104 investigate how parasitism drives adaptive behavioural responses in hosts, which could aid in
105 alleviating parasite burdens. Specifically, we investigate (a) if association times and direct
106 contact patterns between fish change depending on infection status, (b) assess the
107 significance of these behavioural responses in determining successful parasite transmission,
108 and (c) assess how the parasite infrapopulation influences transmission dynamics,
109 irrespective of behaviour.

110

111 **MATERIALS AND METHODS**

112

113 *Ethics statement*

114

115 All applicable institutional and/or national guidelines for the care and use of animals were
116 followed. Procedures and protocols were conducted under UK Home Office license (PPL
117 302876) with approval by the Cardiff University Animal Ethics Committee.

118

119 *Host and parasite origin*

120

121 Experimental Trinidadian guppies (*Poecilia reticulata*) were laboratory-reared descendants
122 of a wild stock caught from the Lower Aripo River, 2012. Fish were initially housed at
123 Exeter University, before being transferred to Cardiff University in October 2014. Here, fish

124 were maintained in 70L dechlorinated water tanks under standard conditions of $24 \pm 1^\circ\text{C}$ on a
125 12 h light: 12 h dark photoperiod (lights on 07:00-19:00), and fed daily with Aquarian
126 tropical fish flakes subsidised with *Daphnia magna* and *Artemia* spp.

127

128 Experimental fish were infected with *Gyrodactylus turnbulli* (strain Gt3), originating from,
129 and subsequently maintained on, an inbred ornamental guppy stock since 1997. A single
130 parasite was isolated onto a naïve ‘culture’ fish, and following successful establishment
131 reproduced forming a highly inbred parasite population. This population has since been
132 maintained under standard conditions in culture pots, each containing a minimum of four
133 naïve culture fish collectively infected with *ca.* 30 *G. turnbulli* worms. Naïve fry were added
134 to the culture pots biweekly to prevent parasite extinction.

135

136 *Experimental design*

137

138 Only female guppies were used during experimental trials due to their increased propensity to
139 shoal compared to males (Griffiths and Magurran, 1998). In the wild, females typically form
140 small shoals (2-20 individuals), between which males move in search of mating opportunities
141 (Croft *et al.* 2004). Thus, by excluding males we eliminated potentially confounding factors
142 associated with mating attempts in influencing parasite transmission. Unfamiliarised female
143 guppies were size-matched according to their standard length (SL; $\pm 4.1\text{mm}$) into pairs ($n =$
144 50), and individually housed in 1L-dechlorinated water under standard conditions. As
145 infection histories of these fish differed, we categorised dyads into five treatments
146 summarised in Table 1. Treatment 1 utilised parasite naïve guppies and formed a control
147 group. During trials these fish were sham infected to account for handling time but never
148 exposed to parasites. Naïve guppies were also used for Treatment 2, which were

149 experimentally infected with a predetermined dose of *Gyrodactylus turnbulli* worms
150 ('Primary infection': see Table 1), and tested 24 h post infection. Treatments 3, 4 and 5
151 comprised dyads that had experienced *G. turnbulli* infection in a previous experiment
152 (ranging from 20-40 worms), but had been clear of parasites for a minimum of three months
153 prior to this experiment. During a trial, one fish in each dyad was experimentally infected
154 with 40 *G. turnbulli* worms ('Secondary infection'), and tested 24 (Treatment 3), 48
155 (Treatment 4) and 72 h (Treatment 5) post-infection.

156

157 Behavioural trials took place in a partitioned glass tank (30 x 30 x 45cm) filled with 7L-
158 dechlorinated water and maintained at $24 \pm 1^{\circ}\text{C}$. A removable translucent barrier separated
159 dyads prior to a trial, allowing visual but not physical contact. The tank was placed in an
160 experimental chamber surrounded by white fabric on three sides to prevent external
161 disturbance, with one side left open for behavioural observations. A 2cm^2 grid attached to the
162 sides of the tank enabled visual estimation of the distances between conspecifics. The arena
163 was lit from above using daylight mimicking strip lights (Sylvania T5 F13W/54-765 G5
164 Luxline Standard Daylight bulb) diffused by white fabric.

165

166 *Experimental procedure*

167

168 Each dyad underwent a two-stage trial comprising behavioural and parasite transmission
169 procedures. On Day 1, dyads were placed into the partitioned experimental tank for a 24 h
170 acclimation period. On Day 2, both guppies were transferred from the tank to individual 1L-
171 dechlorinated water pots using a plastic container. One fish in each dyad was then infected
172 with a predetermined number of *Gyrodactylus turnbulli* worms (see Table 1 for *G. turnbulli*
173 dose and sample sizes). Experimental infections entailed sacrificing a heavily infected culture

174 fish, and placing it in close proximity to a temporarily anaesthetised recipient fish (using
175 0.02% buffered MS222), allowing direct transmission of worms. The infection process was
176 observed continuously under a dissection microscope with fibre optic illumination, and once
177 infected a recipient was revived in 1L-dechlorinated water. It's uninfected conspecific was
178 sham infected to control for handling time and each pair returned to the partitioned tank for a
179 subsequent 24 h, thus ensuring fish were familiar with this environment to minimise
180 exploratory behaviour during a subsequent trial.

181

182 On Day 3, both fish were removed from the partitioned tank, as above, temporarily
183 anaesthetised and screened under a dissection microscope to quantify the starting *G. turnbulli*
184 intensity of a donor, and confirm the uninfected status of their conspecific. The number of
185 individual worms was counted three times to ensure exact intensities were recorded.
186 Variability in *G. turnbulli* start intensity, particularly for treatments 4 and 5 (see Table 1)
187 were observed given the parasites 24 h reproductive potential (Bakke *et al.* 2007). Fish were
188 returned to the partitioned tank for 10 min prior to removal of the translucent barrier, which
189 allowed dyads access to one another. A 10 min behavioural trial began when one guppy
190 crossed the central partition line. During a trial, the proportion of time dyads spent
191 associating was quantified (seconds) using a stopwatch based on direct observation. An
192 association was defined as when fish were \leq four body lengths from one other; a standard
193 measure of shoaling (Pitcher, 1983). Additionally, the number of direct contacts initiated by
194 the infected fish was tallied. Infected guppies exhibit clamped fins (Cable *et al.* 2002), and
195 appear to 'rub-up' against conspecifics, although previously only anecdotal evidence of such
196 behaviour exists (Croft *et al.* 2011; Stephenson *et al.* 2017). This unique behavioural response
197 of infected fish coupled with pathological symptoms of infection made the identity of each
198 fish obvious during a trial. A direct contact was therefore defined as when an infected fish

199 instigated skin-skin contact (typically lasting <1 sec) with an uninfected conspecific, which is
200 particularly important for facilitating *G. turnbulli* transmission (Bakke et al. 2007). Following
201 a behavioural trial, both fish were again individually removed from the partitioned tank,
202 temporarily anaesthetised and screened to quantify successful transmission of individual *G.*
203 *turnbulli* worms

204

205 To assess the significance of *G. turnbulli* infrapopulation intensity on potential *G. turnbulli*
206 transmission dynamics, guppies remained anaesthetised following screening, and were placed
207 in the same petri dish submerged in 1 cm depth dechlorinated water. Using a pipette tip, fish
208 were manipulated so that they were in direct contact with one another. The number of *G.*
209 *turnbulli* worms to transmit from a donor to a recipient fish was then monitored for a 5 min
210 period. At the end of a trial, guppies were chemically treated using 0.1% levamisole and
211 screened clear of infection, on 3 consecutive inspections, before returning to stock tanks.

212

213 *Statistical analysis*

214

215 Statistical analyses were performed in R (3.0.2; R Core Team 2013). Using the *lme4* library
216 (Bates *et al.* 2014), two Generalised Linear Mixed Effects Models (GLMMs), fitted with
217 binomial family and ‘logit’ error structures, were used to assess variables influencing the
218 proportion of time dyads spent associating (the dependent term in both models). The first
219 model included donor *Gyrodactylus turnbulli* intensity, dyad mean SL, treatment, and
220 interactions between each variable, as independent terms. Treatment 1 data was excluded
221 from this model, as there was no possible relationship between the uninfected control dyads
222 and parasite intensity. To address differences in dyad association time between treatments
223 (including Treatment 1), a second GLMM was performed whereby dyad mean SL, treatment

224 and an interaction between these terms were included in a model as independent terms, with
225 dyad association time the dependent term. Parasite intensity was excluded from this model.
226 Both models included dyad ID as a random term to account for repeated measures.

227

228 Using the *glmmADMB* package (Bolker *et al.* 2008) a negative binomial GLMM was used to
229 investigate the effects of dyad mean SL, donor *G. turnbulli* intensity, association time and
230 treatment on the total number of direct contacts observed between dyads, instigated by an
231 infected donor. Interactions between association time x *G. turnbulli* intensity and treatment x
232 *G. turnbulli* intensity were incorporated into the model. A final negative binomial GLMM
233 investigated the significance behavioural trials, transmission trials, dyad mean SL, treatment
234 and donor *G. turnbulli* intensity had on determining total *G. turnbulli* transmission. Dyad ID
235 was incorporated into each model as a random term to account for repeated measures. Models
236 were refined via the sequential removal of non-significant terms to minimise Akaike
237 Information Criterion (Pinheiro and Bates, 2000; Bates *et al.* 2014), and model robustness
238 assessed using residual plots.

239

240 **RESULTS**

241

242 *Behavioural trials*

243

244 Infection with *Gyrodactylus turnbulli* significantly influenced the proportion of time dyads
245 spent associating (GLMM: $z = -7.27$, SE = 0.022, $P < 0.001$), which also differed between
246 experimental treatments (GLMM: $z = -4.33$, SE = 0.189, $P < 0.001$). For fish infected with *G.*
247 *turnbulli* up to 24 h (Treatments 2 & 3), the time dyads spent associating increased with
248 parasite intensity (Fig. 1a). Conversely, for dyads experiencing secondary infections of 48

249 and 72 h, association time decreased as a donor infection intensified (Treatments 4 & 5,
250 respectively: Fig. 1a). Dyad mean SL significantly influenced association time, which also
251 depended on treatment (Dyad mean SL x Treatment interaction, GLMM: $z = -10.48$, SE =
252 0.004, $P < 0.001$). For control dyads and naïve fish enduring a 24 h primary *G. turnbulli*
253 infection (Treatment 2), larger dyads spent more time associating (Fig. 1b). For fish infected
254 with 24, 48 and 72 h secondary infections (Treatments 3, 4 and 5, respectively), the
255 relationship between dyad shoaling time and mean SL was less pronounced (Fig. 1b).

256

257 Association time and *G. turnbulli* intensity significantly influenced the number of direct
258 contacts initiated by an infected donor towards its uninfected conspecific. Specifically, more
259 direct contacts occurred the longer dyads spent associating (GLMM: $z = 1.98$, SE = 0.767, P
260 < 0.005), and as a donors parasite intensity increased (GLMM: $z = 2.80$, SE = 0.029, $P =$
261 0.0309; Fig 2a). However, only on two occasions did successful *G. turnbulli* transmission
262 occur as a consequence of host behaviour.

263

264 *Gyrodactylus turnbulli* transmission

265

266 When investigating the potential for *Gyrodactylus turnbulli* transmission following host
267 behavioural trials (i.e. placing an anaesthetized donor fish in direct contact with an uninfected
268 recipient for a 5 min period), we found that the total number of *G. turnbulli* to transmit was
269 significantly influenced by a donors' parasite intensity (GLMM: $z = 2.09$, SE = 0.006, $P =$
270 0.037). As parasite intensity increased, the number of parasites transmitting to a recipient also
271 increased (Fig. 2b). The duration of infection did not affect total *G. turnbulli* transmission
272 (Treatment main effect: GLMM: $z = 1.08$, SE = 0.10, $P = 0.278$).

273

274 **DISCUSSION**

275

276 Here we show that infected fish significantly increase direct contact rates with conspecifics.

277 As uninfected fish did not perform such behaviour, we speculate that this parasite-driven host

278 behavioural response attempts to reduce parasite burdens. Additionally, we show that

279 association times between fish exposed to secondary *G. turnbulli* infection was less than

280 those experiencing infection for the first time. We speculate that fish build an infection cue

281 repertoire, enabling them to rapidly identify and subsequently avoid infectious conspecifics.

282

283 Our results are consistent with Croft *et al.* (2011), who provided anecdotal evidence of

284 infected guppies initiating direct body contact with conspecifics. Although gyrodactylid

285 infections do cause host behavioural modifications (see Bakke *et al.* 2007), these are merely

286 by-products of infection (e.g. Kolluru *et al.* 2009). Behavioural manipulation by *G. turnbulli*

287 would imply host neurochemical interference (Adamo and Webster, 2013), which has

288 previously never been shown by these ectoparasites. Furthermore, *G. turnbulli* worms did not

289 exhibit a strong transmission propensity during behavioural trials, indicating that increased

290 contact rates are likely an adaptive behavioural response of the host as opposed to parasite

291 manipulation.

292

293 Alleviating ectoparasite burdens often involves hosts participating in interspecific,

294 intraspecific and/or self-grooming behaviours (reviewed in Hart, 2011). Intraspecific

295 grooming is particularly evident in higher animal taxa, most notably mammals such as

296 ungulates. Reciprocal allogrooming in impala (*Aepyceros melampus*), for example,

297 significantly reduced tick infestations by up to 95% that of controls (Mooring *et al.* 1996).

298 Fish also engage in grooming activity, whereby cleaners including gobies, wrasse or shrimps

299 remove clientele ectoparasites, which provides nutritional and health benefits for both parties,
300 respectively (Grutter, 1996; Whiteman and Côté, 2002; Titus *et al.* 2017). Cleaning services
301 are typically concentrated to tropical coral reefs (Côté, 2000), although freshwater
302 intraspecific examples do exist (e.g. Common carp; Soto *et al.* 1994). For fishes that do not
303 engage in cleaning services, alternative parasite removal strategies are employed. For
304 example, physical dislodgement of parasites through skin abrasion against substrata (Urawa,
305 1992), behavioural fever (Reynolds *et al.* 1976; Mohammed *et al.* 2016), or as observed here
306 increasing contact frequencies with uninfected conspecifics in the early stages of infection.
307 The number of direct contacts instigated by infected guppies towards their uninfected
308 conspecifics significantly increased with *G. turnbulli* intensity. Direct contact between hosts
309 governs *G. turnbulli* transmission dynamics (see Johnson *et al.* 2011), and it could be
310 expected that parasite transmission increase linearly with direct contact frequencies. Contrary
311 to our predictions, successful *G. turnbulli* transmission only occurred twice during
312 behavioural trials, irrespective of the number of direct contacts between infected and
313 uninfected fish. This indicates that such behavioural responses adopted by a host were
314 ineffective in reducing parasite burdens, at least in the initial stages of infection.

315

316 Transmission dynamics are mediated by a complexity of factors from both host and parasite
317 perspectives (see Stephenson *et al.* 2017). Ectoparasites adopting a direct transmission
318 strategy risk dislodgment and subsequent mortality. Dislodged *G. turnbulli*, for example,
319 have a maximal off host survival of 31 h at 25°C (Schelkle *et al.* 2013). In the initial stages of
320 infection when the parasite infrapopulation is low, worms do not compete for host resources
321 and therefore the transmission risk outweighs the cost of remaining on an initial host.
322 Although not examined here, in the later stages of infection competition for resources due to
323 a greater parasite density, coupled with activation of the host's immune response (Buchmann

324 and Bresciani, 1998), may prompt parasite transmission away from unfavourable host
325 microhabitats (Boeger *et al.* 2005; Pie *et al.* 2006).

326

327 For parasite naïve controls, dyad association time increased with mean SL. Similarly, this
328 trend was observed for dyads experiencing a 24 h primary *G. turnbulli* infection, although a
329 slight decrease in association time was evident. Larger fish generally have greater shoaling
330 tendencies than smaller, younger individuals (Pitcher *et al.* 1983; Paxton, 1996; Rodgers *et*
331 *al.* 2011). This may be associated with ‘safety in numbers’ as large individuals are more
332 conspicuous to predators. Fish experiencing secondary infections, however, only associated
333 when infection intensities were low. These individuals may have developed a chemical cue
334 repertoire of infection, which overrides their social preferences irrespective of size. During
335 development, individuals imprint on both behavioural and chemical cues of conspecifics to
336 build a ‘phenotypic template’, which is important for social decisions later on in life (see
337 Mateo, 2004). Using these templates, individuals decipher between normal and abnormal
338 cues emitted from conspecifics, to which they can respond accordingly. Such cues important
339 in communicating the infection status of individuals could be associated with a host’s
340 immune response.

341

342 Fish exhibit both innate and acquired immune responses to gyrodactylid infections (e.g. Scott
343 and Robinson, 1984; Scott, 1985; Cable and van Oosterhout, 2007), which are directed to the
344 hosts’ epidermis (Richards and Chubb, 1996). Here, immune by-products including host
345 complement (Buchmann, 1998), changes in mucosal secretion composition (Moore *et al.*
346 1994), and cortisol release into the surrounding water (Stoltze and Buchmann, 2001) translate
347 into chemical cues indicative of infection. Additionally, fish may also perceive excretory
348 compounds of ectoparasites, although to date this remains unknown. Detection of these cues

349 subsequently mediates avoidance behaviours to reduce infection risk, particularly in the late
350 stages of infection (Stephenson and Reynolds 2016). Furthermore, the combination of both
351 innate and acquired immunity during secondary infections could have emitted stronger
352 'infection cues' resulting in a significant reduction in the association times between infected
353 and uninfected fish exposed to 48 and 72 h secondary *G. turnbulli* infections. It should be
354 noted, however, that acquired resistance diminishes post-recovery (Scott, 1985; Cable and
355 van Oosterhout, 2007), and as our experimental fish had previously been uninfected for over
356 3 months, it is unlikely that acquired immune responses of infected fish contributed to the
357 production of cues indicative of infection. Alternatively, the development and retention of a
358 chemical cue repertoire of infection during primary parasite exposure may have been
359 important in instigating evasive fish behaviours during subsequent infection exposure. Such
360 parasite-mediated avoidance behaviour is particularly beneficial considering the severe
361 pathological costs of gyrodactylid infection (reviewed in Bakke *et al.* 2007).

362

363 To conclude, this study shows that in the early stages of infection, parasite-driven
364 behavioural modifications of infected hosts is not an effective strategy for reducing parasite
365 burdens. Such behavioural responses may have greater significance in determining parasite
366 transmission during the later stages of infection, when infra-parasite competition and the
367 host's immune response are more pronounced. However, as infection progresses the benefits
368 may be short lived, particularly if the parasite's reproductive rate outweighs a host's
369 offloading ability. Successful transmission as a consequence of increased contact rates would
370 also be costly to conspecifics, and is counterintuitive to the evolutionary theory of sociality.
371 Although we provide the first quantified example of such behaviour, it may be more
372 widespread within the Animal Kingdom as infected hosts attempt to alleviate their infections.
373 Finally, this study provides evidence that fish exposed to secondary parasite infections may

374 have developed infection repertoires, enabling them to instigate evasive behaviours towards
375 infectious conspecifics sooner than fish experiencing infection for the first time.

376

377 **ACKNOWLEDGEMENTS**

378

379 We thank Darren Croft for providing the original fish for the experiment and Dayna Lea for
380 technical assistance. MR designed the study; MR, EAA and DL collected the data; MR
381 performed statistical analysis and drafted the manuscript; JC made extensive comments on
382 the manuscript.

383

384 **REFERENCES**

385

386 Adamo, S. A., and Webster, J. P. (2013). Neural parasitology: how parasites manipulate host
387 behaviour. *Journal of Experimental Biology*. **216**, 1-2. doi: 10.1242/jeb.082511.

388 Alexander, R. D. (1974). The evolution of social behaviour. *Annual Review of Ecology,*
389 *Evolution and Systematics*. **5**, 325-383. doi: [10.1146/annurev.es.05.110174.001545](https://doi.org/10.1146/annurev.es.05.110174.001545).

390 Bakke, T. A., Cable, J., and Harris, P. D. (2007). The biology of gyrodactylid monogeneans:
391 the “Russian-doll killers”. *Advances in Parasitology*. **64**, 161-378. doi:
392 10.1016/S0065-308X(06)64003-7.

393 Bates, D., Maechler, M., Bolker, B., and Walker, S. (2014). *lme4*: Linear mixed-effects
394 models using Eigen and S4. R package version 1.1-6. url: [http://CRAN.R-project.](http://CRAN.R-project.org/package=lme4)
395 [org/package=lme4](http://CRAN.R-project.org/package=lme4).

396 Bell, D. C., Atkinson, J. S., and Carlson, W. (1999). Centrality measures for disease
397 transmission networks. *Social Networks*. **21**, 1-21. doi: [10.1016/S0378-](https://doi.org/10.1016/S0378-8733(98)00010-0)
398 [8733\(98\)00010-0](https://doi.org/10.1016/S0378-8733(98)00010-0).

399 Boeger, W. A., Kritsky, D. C., Pie, M. R., and Engers, K. B. (2005). Mode of transmission,
400 host switching, and escape from the Red Queen by viviparous gyrodactylids
401 (Monogeneoidea). *Journal of Parasitology*. **91**, 1000-1007. doi: [10.1645/GE-515R.1](https://doi.org/10.1645/GE-515R.1).

402 Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H.,
403 and White, J-S, S. (2008). Generalized linear mixed models: a practical guide for
404 ecology and evolution. *Trends in Ecology and Evolution*. **24**, 127-135.
405 doi:10.1016/j.tree.2008.10.008.

406 Buchmann, K. and Bresciani, J. (1998). Microenvironment of *Gyrodactylus derjavini*:
407 association between mucous cell density and microhabitat selection. *Parasitology*
408 *Research*. **84**, 17-24. doi: 10.1007/s004360050350.

409 Buchmann, K. (1998). Binding and lethal effect of complement from *Oncorhynchus mykiss*
410 on *Gyrodactylus derjavini* (Platyhelminthes: Monogenea). *Diseases of Aquatic*
411 *Organisms*. **32**, 195-200. doi: [10.3354/dao032195](https://doi.org/10.3354/dao032195).

412 Cable, J., Scott, E. C. G., Tinsley, R. C. and Harris, P. D. (2002). Behavior favoring
413 transmission in the viviparous monogenean *Gyrodactylus turnbulli*. *Journal of*
414 *Parasitology*. **88**, 183-184. doi: 10.1645/0022-
415 3395(2002)088[0183:BFTITV]2.0.CO;2.

416 Cable, J., and van Oosterhout, C. (2007). The role of innate and acquired resistance in two
417 natural populations of guppies (*Poecilia reticulata*) infected with the ectoparasite
418 *Gyrodactylus turnbulli*. *Biological Journal of the Linnean Society*. **90**, 647-655. doi:
419 10.1111/j.1095-8312.2006.00755.x.

420 Côté, I. M., and Poulin, R. (1995). Parasitism and group size in social animals: a meta-
421 analysis. *Behavioural Ecology*. **6**, 159-165. doi: [10.1093/beheco/6.2.159](https://doi.org/10.1093/beheco/6.2.159).

422 Côté, I. M. (2000). Evolution and ecology of cleaning symbiosis in the sea. *Oceangeographic*
423 *Marine Biology*. **38**, 311-355. doi: 10.1201/b12589.

424 Curtis, V. A. (2014). Infection-avoidance behaviour in humans and other animals. *Trends in*
425 *Immunology*. **35**, 457-464. doi: [10.1016/j.it.2014.08.006](https://doi.org/10.1016/j.it.2014.08.006).

426 Craft, M. E. (2015). Infectious disease transmission and contact networks in wildlife and
427 livestock. *Philosophical Transactions of the Royal Society B: Biological Sciences*.
428 **370**: 20140107. doi: 10.1098/rstb.2014.0107.

429 Croft, D. P., Krause, J., and James, R. (2004). Social networks in the guppy (*Poecilia*
430 *reticulata*). *Proceedings of the Royal Society of London B: Biological Sciences*. **271**,
431 516-519. doi: 10.1098/rsbl.2004.0206.

432 Croft, D. P., Edenbrown, M., Darden, S. K., Ramnarine, I. W., van Oosterhout, C., and
433 Cable, J. (2011). Effect of gyrodactylid ectoparasites on host behaviour and social
434 network structure in guppies *Poecilia reticulata*. *Behavioural Ecology and*
435 *Sociobiology*. **65**, 2219-2227. doi: [10.1007/s00265-011-1230-2](https://doi.org/10.1007/s00265-011-1230-2).

436 Danon, L., Ford, A. P., House, T., Jewell, C. P., Keeling, M. J., Roberts, G. O., Ross, J. V.,
437 and Vernon, M. C. (2011). Networks and the epidemiology of infectious disease.
438 *Interdisciplinary Perspectives on Infectious Diseases*. **2011**, 284909. doi:
439 [10.1155/2011/284909](https://doi.org/10.1155/2011/284909).

440 Duncan, P., and Vigne, N. (1979). Effect of group-size in horses on the rate of attacks by
441 blood-sucking flies. *Animal Behaviour*. **27**, 623-625. doi: 0.1016/0003-
442 3472(79)90201-X,

443 Faria, P. J., van Oosterhout, C., and Cable, J. (2010). Optimal release strategies for captive-
444 bred animals in reintroduction programs: experimental infections using the guppy as a
445 model organism. *Biological Conservation*. **143**, 35-41. doi:
446 [10.1016/j.biocon.2009.06.002](https://doi.org/10.1016/j.biocon.2009.06.002).

447 Glaser, R., and Kiecolt-Glaser, J. K. (2005). Stress-induced immune dysfunction:
448 implications for health. *Nature Reviews Immunology*. **5**, 243-251. doi:
449 10.1038/nri1571.

450 Griffiths, S. W., and Magurran, A. E. (1998). Sex and schooling behaviour in the Trinidadian
451 guppy. *Animal Behaviour*. **56**, 689-693. doi: [10.1006/anbe.1998.0767](https://doi.org/10.1006/anbe.1998.0767).

452 Grutter, A. (1996). Parasite removal rates by the cleaner wrasse *Labroides dimidiatus*. *Inter-*
453 *Research Marine Ecology Progress Series*. **130**, 61-70. doi: 10.3354/meps130061.

454 Hart, B. L. (2011). Behavioural defences in animals against pathogens and parasite: parallels
455 with the pillars of medicine in humans. *Philosophical Transactions of the Royal*
456 *Society B: Biological Sciences*. **366**, 3406-3417. doi: 10.1098/rstb.2011.0092.

457 Heinze, J., and Walter, B. (2010). Moribund ants leave their nests to die in social isolation.
458 *Current Biology*. **20**, 249-252. doi: [10.1016/j.cub.2009.12.031](https://doi.org/10.1016/j.cub.2009.12.031).

459 Johnson, M. B., Lafferty, K. D., van Oosterhout, C., and Cable, J. (2011). Parasite
460 transmission in social interacting hosts: monogenean epidemics in guppies. *PLoS*
461 *ONE*. **6**: e22634. doi: [10.1371/journal.pone.0022634](https://doi.org/10.1371/journal.pone.0022634).

462 Kiesecker, J. M., Skelly, D. K., Beard, K. H., and Preisser, E. (1999). Behavioural reduction
463 of infection risk. *Proceedings of the National Academy of Sciences USA*. **96**, 9165-
464 9168. doi: 10.1073/pnas.96.16.9165.

465 Kolluru, G. T., Grether, G. F., Dunlop, E., South, S. H. (2009). Food availability and parasite
466 infection influence mating tactics in guppies (*Poecilia reticulata*). *Behavioural*
467 *Ecology*. **20**, 131–137. doi: [10.1093/beheco/arn124](https://doi.org/10.1093/beheco/arn124).

468 Koprivnikar, J., and Penalva, L. (2015). Lesser of two evils? Foraging choices in response to
469 threats of predation and parasitism. *PLoS One*. **10**: e0116569. doi:
470 [10.1371/journal.pone.0116569](https://doi.org/10.1371/journal.pone.0116569).

471 Krause, J., and Ruxton, G. D. (2002). *Living in groups*. Oxford University Press, Oxford,
472 UK.

473 Lloyd-Smith, J. O., Schreiber, S. J., Kopp, P. E., and Getz, W. M. (2005). Superspreading
474 and the effect of individual variation on disease emergence. *Nature*. **438**, 355-359.
475 doi: 10.1038/nature04153.

476 Mateo, J. M. (2004). Recognition systems and biological organization: the perception
477 component of social recognition. *Annales Zoologici Fennici*. **41**, 729-745.

478 Mohammed, R. S., Reynolds, M., James, J., Williams, C., Mohammed, A., Ramsubhag, A.,
479 van Oosterhout, C., and Cable, J. (2016). Getting into hot water: sick guppies frequent
480 warmer thermal conditions. *Oecologia*. **181**, 911-917. doi: 10.1007/s00442-016-3598-
481 1.

482 Moore, M. M., Kaattari, S. L., and Olson, R. E. (1994). Biologically active factors against the
483 monogenetic trematodes *Gyrodactylus stellatus* in the serum and mucus of infected
484 juvenile English soles. *Journal of Aquatic Animal Health*. **6**, 93-100. doi:
485 [10.1577/1548-8667\(1994\)006](https://doi.org/10.1577/1548-8667(1994)006).

486 Mooring, M. S., and Hart, B. L. (1992). Animal grouping for protection from parasites:
487 selfish herd and ecounter-dilution effects. *Behaviour*. **123**, 173-193. doi:
488 10.1163/156853992X00011.

489 Mooring, M. S., McKenzie, A. A., and Hart, B. L. (1996). Grooming in impala: role of oral
490 grooming in removal of ticks and effects of ticks in increasing grooming rate.
491 *Physiology and Behaviour*. **59**, 965-971. doi: [10.1016/0031-9384\(95\)02186-8](https://doi.org/10.1016/0031-9384(95)02186-8).

492 Patterson, J. E., and Ruckstuhl, K. E. (2013). Parasite infection and host group size: a meta-
493 analytical review. *Parasitology*. doi: 10.1017/S0031182012002259.

494 Paxton, C. G. (1996). Isolation and the development of shoaling in two populations of the
495 guppy. *Journal of Fish Biology*. **49**, 514-520. doi: 10.1111/j.1095-
496 8649.1996.tb00046.x

497 Pitcher, T. J. (1983). Heuristic definitions of shoaling behaviour. *Animal behaviour*. **31**, 611-
498 163. doi: 10.1016/S0003-3472(83)80087-6.

499 Pitcher, T. J., Magurran, A. E., and Allan, J. R. (1983). Shifts of behaviour with shoal size in
500 cyprinids. *Proceedings of the British Freshwater Fisheries Conference*. **3**, 220-228.

501 Pie, M. R., Engers, K. B., and Boeger, W. A. (2006). Density-dependent topographical
502 specialization in *Gyrodactylus anisopharynx* (Monogenea, Gyrodactylidae):
503 Boosting transmission or evading competition? *Journal of Parasitology*. **92**, 459-463.
504 doi: [10.1645/GE-641.1](https://doi.org/10.1645/GE-641.1).

505 Pinheiro, J. C., and Bates, D. M. (2000). Linear Mixed-Effects Models: Basic Concepts
506 and Examples. In: *Mixed-Effects Models in S and S-PLUS*. Statistics and
507 Computing. Springer, New York, NY. doi: 10.1007/0-387-22747-4_1.

508 Poirotte, C., Massol, F., Herbert, A., Willaume, E., Bomo, P. M., Kappeler, P. M., and
509 Charpentier, M. J. E. (2017). Madrills use olfaction to socially avoid parasitized
510 conspecifics. *Science Advances*. **3**, e1601721. doi: 10.1126/sciadv.1601721.

511 Poulin, R. (2000). Manipulation of host behaviour by parasites: a weakening paradigm?
512 *Proceedings of the Royal Society of London B: Biological Sciences*. **267**, 787-792.
513 doi: [10.1098/rspb.2000.1072](https://doi.org/10.1098/rspb.2000.1072).

514 Proudfoot, K, and Habing, G. (2015). Social stress as a cause of diseases in farm animals:
515 Current knowledge and future direction. *The Veterinary Journal*. **206**, 15-21. doi:
516 10.1016/j.tvjl.2015.05.024.

517 R Development Core Team. (2009). R: A language and environment for statistical
518 computing: the R Foundation for Statistical Computing. Vienna, Austria.

519 Rätti, O., Ojanen, U., and Helle, P. (2006). Increasing group size dilutes black fly attack rate
520 in Black Grouse. *Ornis Fennica*. **83**, 86-90.

521 Reynolds, W. W., Casterlin, M. E., and Covert, J. B. (1976). Behavioural fever in teleost fish.
522 *Nature*. **259**, 41-42. doi: 10.1038/259041a0.

523 Richards, G. R., and Chubb, J. (1996). Host response to initial and challenge infections,
524 following treatment, of *Gyrodactylus bullatarudis* and *G. turnbulli* (Monogenea) on
525 the guppy (*Poecilia reticulata*). *Parasitology Research*. **82**, 242-247. doi:
526 10.1007/s004360050103.

527 Rodgers, G. M., Ward, J. R., Askwith, B., and Morrell, L. J. (2011). Balancing the dilution
528 and oddity effects. Decisions depend on body size. *PLoS ONE*. **6**, e14819. doi:
529 [10.1371/journal.pone.0014819](https://doi.org/10.1371/journal.pone.0014819).

530 Rueppell, O., Hayworth, M. K., and Ross, N. P. (2010). Altruistic self-removal of health-
531 compromised honey bee workers from their hive. *Journal of Evolutionary Biology*.
532 **23**, 1538-1546. doi: [10.1111/j.1420-9101.2010.02022.x](https://doi.org/10.1111/j.1420-9101.2010.02022.x).

533 Sansom, A., Lind, J., and Cresswell, W. (2009). Individual behaviour and survival: the roles
534 of predator avoidance, foraging success, and vigilance. *Behavioural Ecology*. **20**,
535 1168-1174. doi: [10.1093/beheco/arp110](https://doi.org/10.1093/beheco/arp110).

536 Schneider, S. A., Scharffetter, C., Wagner, A. E., Boesch, C., Bruchhaus, I., Rimbach, G.,
537 and Roeder, T. (2016). Social stress increases the susceptibility to infection in the ant
538 *Harpegnathos saltator*. *Scientific Reports*. **6**: 25800. doi: [10.1038/srep25800](https://doi.org/10.1038/srep25800).

539 Scott, M. E., and Robinson, M. A. (1984). Challenge infections of *Gyrodactylus bullatarudis*
540 (Monogenea) on guppies, *Poecilia reticulata* (Peters), following treatment. *Journal of*
541 *Fish Biology*. **24**, 581-586. doi: 10.1111/j.1095-8649.1984.tb04828.x.

542 Scott, M. E. (1985). Dynamics of challenge infection of *Gyrodactylus bullatarudis* Turnbull
543 (Monogenea) on guppies, *Poecilia reticulata* (Peters). *Journal of Fish Biology*. **8**,
544 495-503. doi: 10.1111/j.1365-2761.1985.tb00964.x.

545 Schelkle, B., Snellgrove, D., and Cable, J. (2013). *In vitro* and *in vivo* efficacy of garlic
546 compounds against *Gyrodactylus turnbulli* infecting the guppy (*Poecilia reticulata*).
547 *Veterinary Parasitology*. **198**, 96-101. doi: [10.1016/j.vetpar.2013.08.027](https://doi.org/10.1016/j.vetpar.2013.08.027).

548 Sherman, P. W., Lacey, E. E., Reeve, H. K., and Keller, L. (1995). The eusociality
549 continuum. *Behavioural Ecology*. **6**, 102-108. doi: 10.1093/beheco/6.1.102.

550 Soto, C. G., Zhang, J. S., and Shi, Y. H. (1994). Intraspecific cleaning behaviour in *Cyprinus*
551 *carpio* in aquaria. *Journal of Fish Biology*. **44**, 172-174. doi: 10.1111/j.1095-
552 8649.1994.tb01594.x.

553 Stephenson, J. F., and Reynolds, M. (2016). Imprinting can cause a maladaptive preference
554 for infectious conspecifics. *Biology Letters*. **12**: 20160020. doi:
555 10.1098/rsbl.2016.0020.

556 Stephenson, J. F., Young, K. A., Fox, J., Jokela, J., Cable, J., and Perkins, S. E. (2017). Host
557 heterogeneity affects both parasite transmission to and fitness on subsequent hosts.
558 *Philosophical Transactions of the Royal Society B: Biological Sciences*. **372**,
559 20160093. doi: 10.1098/rstb.2016.0093.

560 Stoltze, K., and Buchmann, K. (2001). Effect of *Gyrodactylus derjavini* infections on cortisol
561 production in rainbow trout fry. *Journal of Helminthology*. **75**, 291-294. doi:
562 10.1079/JOH200157.

563 Titus, B. M., Vondriska, C., and Daly, M. (2017). Comparative behavioural observations
564 demonstrate the ‘cleaner’ shrimp *Periclimenes yucatanicus* engages in true symbiotic
565 cleaning interactions. *Royal Society Open Science*. **4**: 170078. doi:
566 [10.1098/rsos.170078](https://doi.org/10.1098/rsos.170078).

567 Urawa, S. (1992). *Trichodina truttae* Mueller 1937 (Ciliophora, Peritrichida) on juvenile
568 chum salmon (*Oncorhynchus keta*): pathogenicity and host–parasite interactions. *Fish*
569 *Pathology*. **27**, 29–37. doi: 10.3147/jsfp.27.29.

570 Ward, A. J. W., Duff, A. J., Krause, J., and Barber, I. (2005). Shoaling behaviour of
571 sticklebacks infected with the microsporidian parasite, *Glugea anomala*.
572 *Environmental Biology of Fishes*. **72**, 155-160. doi: 10.1007/s10641-004-9078-1.

573 Wey, T., Blumstein, D. T., Shen, W., and Jordán, F. (2008). Social network analysis of
574 animal behaviour: a promising tool for the study of sociality. *Animal Behaviour*. **75**,
575 333-344. doi: [10.1016/j.anbehav.2007.06.020](https://doi.org/10.1016/j.anbehav.2007.06.020).

576 Whitehead, H. (1997). Analysing animal social structure. *Animal Behaviour*. **53**, 1053-1067.
577 doi: [10.1006/anbe.1996.0358](https://doi.org/10.1006/anbe.1996.0358).

578 Whiteman, E. A., and Côté, I. M. (2002). Cleaning activity of two Caribbean cleaning gobies:
579 intra- and interspecific comparisons. *Journal of Fish Biology*. **60**, 1443-1458. doi:
580 [10.1111/j.1095-8649.2002.tb02439.x](https://doi.org/10.1111/j.1095-8649.2002.tb02439.x).

581

582

583

584

585

586

587

588

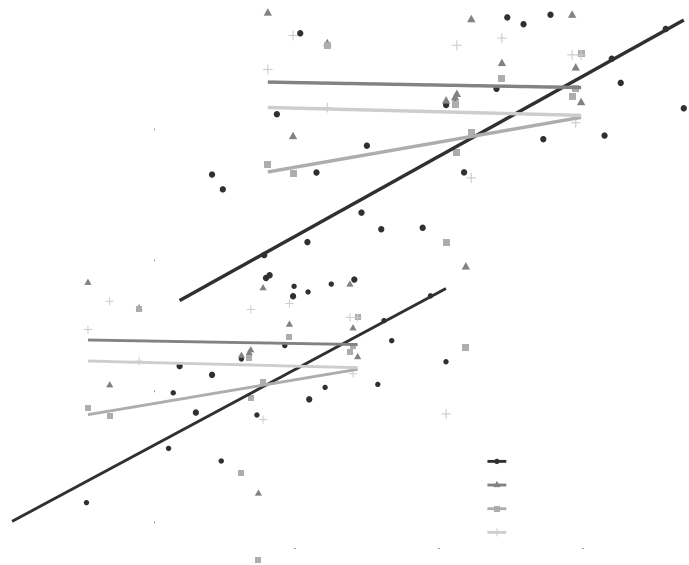
589

590

591

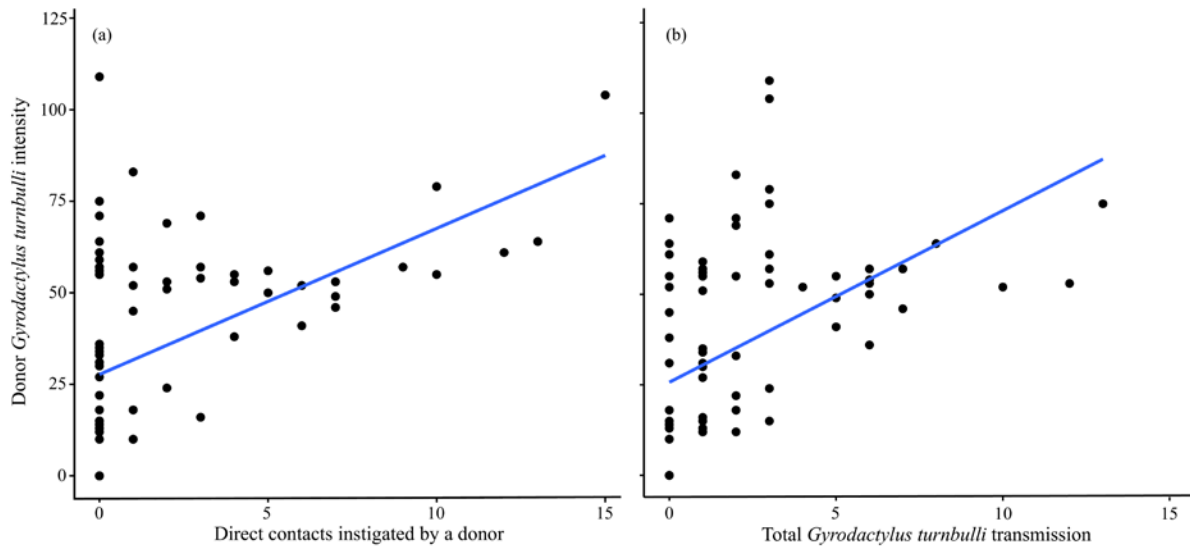
592 **TABLE 1.** A summary of the experimental treatments (1-5) including *Gyrodactylus*
 593 *turnbulli* exposure status (primary or secondary infection), dyad sample size (n), *G. turnbulli*
 594 dose administered and infection time before a behavioural trial.
 595

Treatment	Primary or secondary <i>G. turnbulli</i> infection	Dyad sample size (n)	<i>G. turnbulli</i> intensity dose	Infection period (h)
1 (control)	NA	17	NA	NA
2	Primary	15	10 worms	24
		10	20 worms	
		6	40 worms	
3	Secondary	12	All 40 worms	24
4	Secondary	12	Range: 41-72 worms	48
5	Secondary	10	Range: 36-90 worms	72



596

597 **FIGURE 1.** The relationship between the proportion of time dyads spent shoaling and (a)
 598 *Gyrodactylus turnbulli* intensity of the donor fish, and (b) dyad mean standard length (mm).
 599 The solid lines represent the regression between the proportion of time dyads spent
 600 associating on (a) donor *G. turnbulli* intensity, and (b) dyad mean standard length.



601
 602
 603
 604
 605
 606
 607
 608

FIGURE 2. Positive association between donor *Gyrodactylus turnbulli* intensity (a) the number of direct contacts instigated by an infected donor fish, and (b) the number of parasites to transmit to an uninfected recipient over a 5-minute duration when both fish were anaesthetized and placed in direct contact. The solid line represents the regression between a donor's *G. turnbulli* intensity on (a) the number of direct contacts instigated by a donor, and (b) the number of worms transmitted to an anaesthetized recipient host.