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

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25 **SUMMARY**

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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

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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 ± 1°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; ± 4.1mm) 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 $24 \pm 1°C$. 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 ≤ 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

DISCUSSION

Here we show that infected fish significantly increase direct contact rates with conspecifics. As uninfected fish did not perform such behaviour, we speculate that this parasite-driven host behavioural response attempts to reduce parasite burdens. Additionally, we show that association times between fish exposed to secondary *G. turnbulli* infection was less than those experiencing infection for the first time. We speculate that fish build an infection cue repertoire, enabling them to rapidly identify and subsequently avoid infectious conspecifics.

Our results are consistent with Croft *et al.* (2011), who provided anecdotal evidence of infected guppies initiating direct body contact with conspecifics. Although gyrodactylid infections do cause host behavioural modifications (see Bakke *et al.* 2007), these are merely by-products of infection (e.g. Kolluru *et al.* 2009). Behavioural manipulation by *G. turnbulli* would imply host neurochemical interference (Adamo and Webster, 2013), which has previously never been shown by these ectoparasites. Furthermore, *G. turnbulli* worms did not exhibit a strong transmission propensity during behavioural trials, indicating that increased contact rates are likely an adaptive behavioural response of the host as opposed to parasite manipulation.

Alleviating ectoparasite burdens often involves hosts participating in interspecific, intraspecific and/or self-grooming behaviours (reviewed in Hart, 2011). Intraspecific grooming is particularly evident in higher animal taxa, most notably mammals such as ungulates. Reciprocal allogrooming in impala (*Aepyceros melampus*), for example, significantly reduced tick infestations by up to 95% that of controls (Mooring *et al*. 1996). 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

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378

383

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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 *G. turnbulli* infection	Dyad sample size (n)	*G. turnbulli* 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
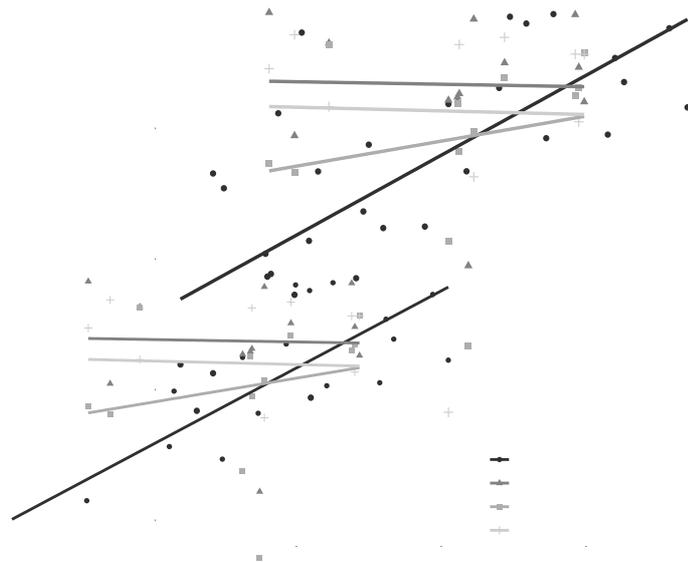
FIGURE 1. The relationship between the proportion of time dyads spent shoaling and (a) *Gyrodactylus turnbulli* intensity of the donor fish, and (b) dyad mean standard length (mm). The solid lines represent the regression between the proportion of time dyads spent associating on (a) donor *G. turnbulli* intensity, and (b) dyad mean standard length.
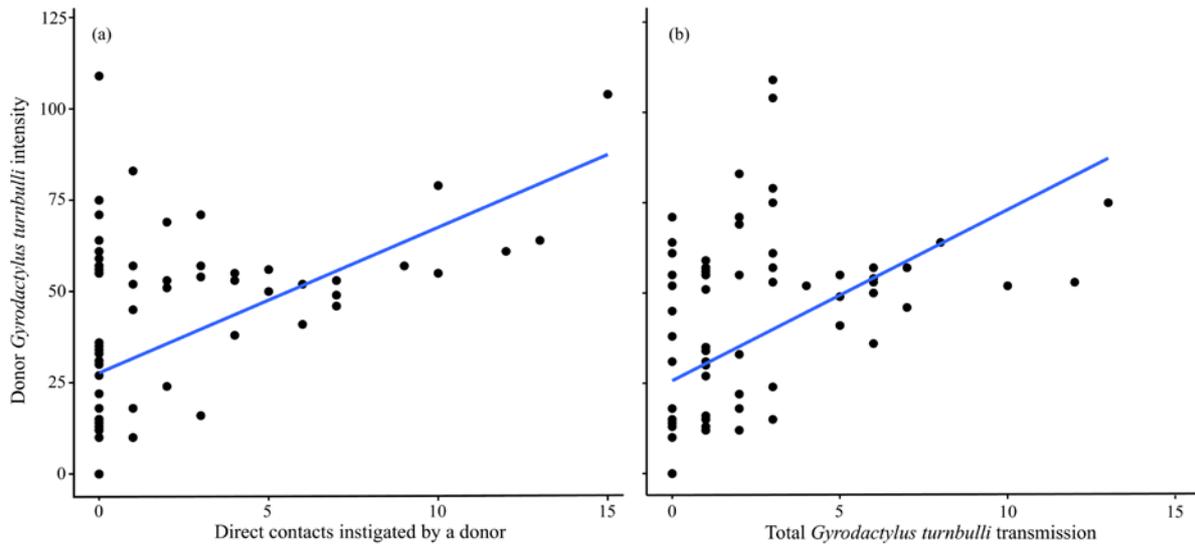
601
602
603 **FIGURE 2.** Positive association between donor *Gyrodactylus turnbulli* intensity (a) the number of
604 direct contacts instigated by an infected donor fish, and (b) the number of parasites to transmit to an
605 uninfected recipient over a 5-minute duration when both fish were anaesthetized and placed in direct
606 contact. The solid line represents the regression between a donor's *G. turnbulli* intensity on (a) the
607 number of direct contacts instigated by a donor, and (b) the number of worms transmitted to an
608 anaesthetized recipient host.