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Interactions between males guppies facilitates the transmission of the monogenean ectoparasite *Gyrodactylus turnbulli*

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HIGHLIGHTS

- ▶ Social behaviour of animals can influence disease dynamics.
- ▶ Transmission of gyrodactylid ectoparasites occurs during male–male interactions.
- ▶ *Gyrodactylus turnbulli* infection is governed by the level of social contact between fish.

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ABSTRACT

In a previous study we found that female guppies shoaled more than males and that there was greater transmission of the ectoparasite *Gyrodactylus turnbulli* between females. Here, to test for a possible sex bias in parasite transmission, we conducted a similar experiment on single sex shoals of male and female guppies, observing host behaviour before and after the introduction of an infected shoal mate. The initial parasite burden was considerably lower in the present experiment (30 worms versus >100 worms previously) and we used a different stock of ornamental guppies (Green Cobra variety versus a Tuxedo hybrid previously). Contrary to our previous finding, males aggregated significantly more than females. Males performed 'sigmoid' displays towards each other, a courtship behaviour that is more generally directed towards females. Due to the high rate of male–male interactions, parasite transmission was 10 times higher between males than between females. Furthermore, shoaling intensity was highest for the most parasitised fish indicating that these infected fish were not avoided by non-parasitised conspecifics. These studies show that certain social behaviours including shoaling and courtship displays, appear to facilitate the transmission of gyrodactylid parasites.

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1. Introduction

Formation of social groups is a common aspect of animal behaviour (e.g. Bertram, 1978) and is based on individuals evaluating the relative profitability of joining, leaving or staying with others due to constantly changing trade-offs between feeding opportunities and predation pressures (Pitcher and Parrish, 1993). Parasitism has been implicated as a potential risk factor impinging on group formation and laboratory experiments indicate that fish can use parasite-infection status as a cue in active shoal choice (Dugatkin et al., 1994; Krause and Godin, 1996). Most previous studies have assessed the impact of indirectly transmitted endoparasites on fish shoaling behaviour and indicated that fish avoid infected conspecifics (review in Barber et al., 2000). We conducted similar experiments using a gyrodactylid-guppy system and predicted greater

avoidance of infected conspecifics with this directly transmitted ectoparasite (Richards et al., 2010; Croft et al., 2011). This did occur with wild fish (Croft et al., 2011), but there was no apparent change in the behaviour of ornamental guppies in single sex shoals after the introduction of a gyrodactylid infected host (Richards et al., 2010). However, because female-only shoals aggregated more than male shoals (as previously demonstrated by Griffiths and Magurran, 1998), there was greater transmission between females than the equivalent groups of males (Richards et al., 2010).

In the wild, shoals of female-only guppies are common, whereas males tend to be more solitary or are found in mixed sex shoals (Magurran, 2005). However, male–male interactions are still commonly observed, particularly in populations that are not heavily female biased (Pettersson et al., 2004). In the ornamental trade, tropical fish (especially livebearers like guppies) are often kept in single sex tanks so investigating the effect of host sex on parasite transmission is particularly important for captive fish stocks. The aim of the current study is to assess

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how the transmission of *Gyrodactylus turnbulli* is affected by the conspecific interactions between males by comparing it with between-female transmission.

2. Materials and methods

Ornamental guppies ($n = 120$) with a phenotype similar to the Green Cobra variety were purchased from a UK commercial supplier in 2007. The fish were already infected with *G. turnbulli* (identified according to Harris et al., 1999) and were subsequently treated with 0.2% levamisole to remove all parasites (see Schelkle et al., 2009) and then left to habituate in the aquarium for at least three months before use. The fish were maintained under a 12 h light: 12 h dark lighting regime in mixed-sex groups (approximately 1 male to 5 females) in $45 \times 45 \times 120$ cm aquaria, and fed a diet of flakes (Aquarian®) and frozen bloodworm. An isogenic strain of *G. turnbulli* (strain Gt3), originally isolated from petshop guppies in 1997, was used for all infections. All experiments were conducted at 25 ± 1 °C between November 2007 and February 2008.

The experimental design has previously been described by Richards et al., (2010). All fish within a tank were size matched within 2–3 mm, with animals across tanks ranging in size from 22 to 30 mm. Briefly, single sex groups of male or female guppies (6 individuals per group with 10 replicate groups) were placed in test aquaria ($40 \times 60 \times 30$ cm), and allowed to acclimatise for 5 d. All fish within an experimental shoal were taken from the same stock tank to ensure similar levels of familiarity. A single guppy in each tank, recognisable by its colour patterns and/or shape, was randomly assigned as the focal fish. An observer tested reliability of accurately identifying the focal fish and if there was any difficulty in distinguishing individuals this shoal was discarded from the experiment. After acclimatisation, the shoaling behaviour of each group was observed once daily for 3 consecutive days ($t =$ days 1–3).

All behavioural observations lasted 15 min per group (5 min in total for each shoaling behaviour parameter). In total, 10 measurements of nearest neighbour distance were made for each focal fish, and for one, randomly chosen, non-focal fish per tank. A further 10 measurements of shoal size were recorded, by counting the number of fish in the largest shoal at the time of observation. The time interval between each of these measures was 30 s. The time spent shoaling by both focal and a random non-focal fish was also measured over 5 min. Horizontal and vertical lines drawn every 2 cm on three sides (back and two sides) of each test aquaria facilitated the estimation of between-individual distances, as all shoaling behaviour measurements were evaluated in three-dimensional space. Shoal members were defined as fish within 4 body lengths of one another (Pitcher, 1983).

At the end of day 3, all fish were removed from the test aquaria and kept individually in 1 l containers while the focal fish was infected with *G. turnbulli*. Each focal fish was anaesthetised in turn using 0.02% MS222 and brought directly into contact with a euthanized infected donor fish within a small glass Petri dish containing dechlorinated water. The manipulation was conducted using a stereo-microscope with fibre optic illumination. The focal fish was removed after approximately 30 worms had transferred. This parasite burden is non-lethal (van Oosterhout et al., 2008), and albeit high, fish with a similar parasite burden have been found in the wild (van Oosterhout et al., 2007). Success of parasite transfer was estimated after 24 h by confining each focal fish in a crystallising dish (5 cm dia.) containing dechlorinated water on the stage of a stereo-microscope and counting the number of parasites (mean \pm SE = 30 ± 14 worms/fish). All non-focal fish were sham infected under anaesthetic using a similar procedure.

Following infection, all fish were returned to their test tank ($t =$ day 4), and the observations on shoaling behaviour (shoal size, nearest neighbour distance, time spent shoaling) were repeated for a further 3 consecutive days. At the end of each trial ($t =$ day 5, 6 and 7) the extent of within-shoal parasite transmission was assessed by recording the number and position of parasites on each individually anaesthetised fish. No fish deaths occurred during the experiment but 2 fish presented with clamped fins (pathology characteristic of *G. turnbulli* infections) on days 6 and 7. All animal work was approved by Cardiff University Ethics Committee and UK Home Office regulations (under licence PPL 30/2357).

2.1. Statistical analyses

Data were natural log-transformed to achieve normality (established using Anderson–Darling tests) and homogeneity of variances (using a Bartlett's test). A Repeated Measures ANOVA was used to analyse whether differences in the three parameters of shoaling behaviour were explained by the day of the experiment, sex and infection status of the guppy. Day of experiment ('Day') was used as a covariate and infection status ('Parasitised') was crossed with sex ('Sex') as factors. To quantify parasite population growth during the experiment, all guppies were assessed for parasite burdens at the end of the 3-day infection period, with differences in initial and final parasite burdens assessed using Kruskal–Wallis tests. Comparisons between males and females in their ability to spread infection to conspecifics were tested using Chi-square analysis. A binary logistic regression analysis (logit) was used with a dichotomous dependent variable, infected or not infected (coded as '1' and '0', respectively), to test whether the infection status of fish at the end of the experimental period was associated with initial parasite load of focal fish ('Gyrostart'), parasite population growth ('Gyrogrow') and sex ('Sex') of the guppy. The model uses 'Sex' as a fixed factor crossed with 'Gyrostart' or 'Gyrogrow' as covariate. For all multivariate analyses, a backwards stepwise elimination of non-significant factors was used to reach a final model. All analyses were performed in Minitab 15.

3. Results

Male guppies had closer and more prolonged contact with each other than did females. Male–male interactions were characterised by typical courtship behaviour, with male guppies regularly performing 'sigmoid' displays (Baerends et al., 1955) directed to other males. In particular, male guppies formed larger groups than females and showed a significantly larger 'average shoal size' (Repeated measures ANOVA: $F_{1,135} = 15.65$, $P = 0.003$). Focal and non-focal male guppies also spent a significantly longer time shoaling than their respective female counterparts (Focal guppies: $F_{1,135} = 35.65$, $P < 0.001$; Non-focal guppies: $F_{1,135} = 22.41$, $P = 0.001$) (Fig. 1A–C). Furthermore, focal males (mean \pm SE 8.6 ± 0.6 cm) had a significantly shorter 'nearest neighbour distance' than focal females (9.7 ± 0.5 cm), as did non-focal males (6.7 ± 0.3 cm) compared to non-focal females (8.6 ± 0.4 cm) (Focal males: $F_{1,135} = 9.00$, $P = 0.015$; Non-focal males: $F_{1,135} = 11.64$, $P = 0.008$). Hence, these results indicate that in this study, males grouped closer together and for longer than females.

There was a significant effect of parasitism on both male and female focal fish. Parasitised fish shoaled significantly more than their uninfected counterparts, forming tighter shoals with a shorter 'nearest neighbour distance' ($F_{1,135} = 27.91$, $P = 0.001$). These parasitised fish also spent more time shoaling ($F_{1,135} = 5.81$, $P = 0.039$). Surprisingly, the (initially uninfected) non-focal fish did not change their shoaling behaviour in the presence of an in-

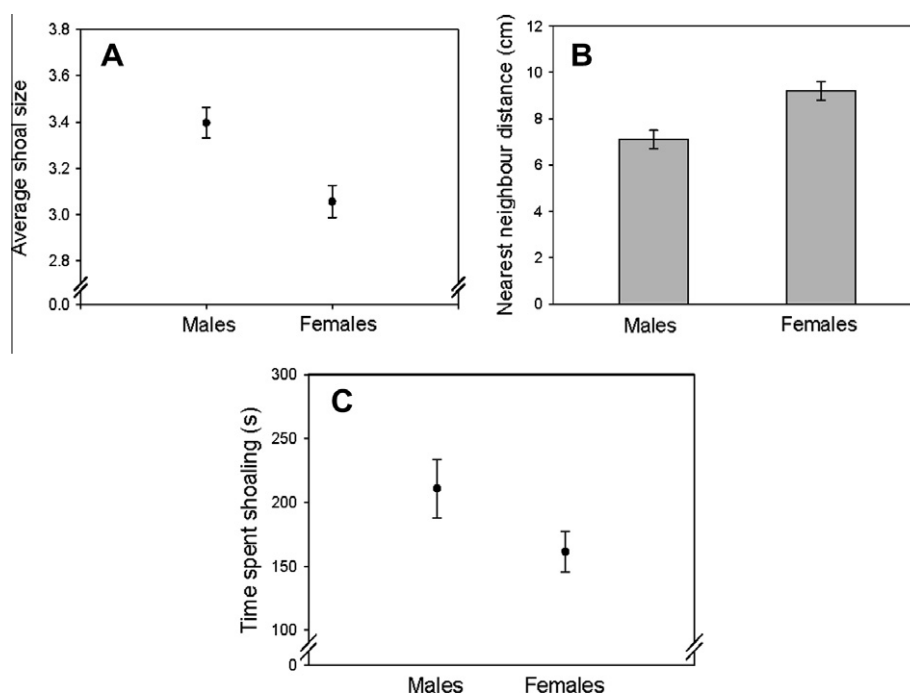


Fig. 1. Mean \pm SE (A) shoal size, (B) distance between nearest neighbours, (C) total time spent shoaling, for male and female guppies (focal and non-focal fish combined).

Table 1

Binary logistic regression for infection status (0 – clean, 1 – infected) with sex of the host ('Sex') as a factor crossed with the initial tank burden ('GyroStart') as covariate.

Predictor	Coef	StDev	Z	P	Odds ratio	95% CI
Constant	-1.5754	0.6836	-2.30	0.021		
Sex	2.3273	0.9889	2.35	0.019	10.25	1.48–71.21
GyroStart	0.0565	0.0249	2.27	0.023	1.06	1.01–1.11
Sex * GyroStart	-0.0646	0.0314	-2.05	0.040	0.94	0.88–1.00

infected conspecific (all 3 measures of shoaling behaviour, Repeated measures ANOVA: $P > 0.1$).

There was no difference between focal males (mean \pm SE 34 ± 4.6 worms/host) and females (mean \pm SE 27 ± 4.6) in their initial parasite loads (Kruskal–Wallis: $H = 1.23$, d.f. = 1, $P = 0.266$). Over the 3-day infection period, focal males experienced (a marginally) significant higher parasite population growth than focal females ($F_{1,135} = 4.51$, $P = 0.049$). Furthermore, focal males had a significantly greater chance of transferring parasites to conspecifics than females in this time (Logistic regression: $Z = -2.05$, $P = 0.040$) (Table 1). In fact, non-focal male guppies were 10 times as likely to acquire an infection compared to non-focal females ($Z = 2.35$, $P = 0.019$).

4. Discussion

The degree of contact between single sex shoals of male and female guppies affected the transmission of the ectoparasite, *G. turnbulli*. Remarkably, male guppies had more frequent and more prolonged contact with each other than females had with each other. Consequently, males were significantly more likely to become infected and transmit the parasite to conspecifics. This is in contrast to our previous study (Richards et al., 2010) where female guppies shoaled significantly more than males, and as a consequence experienced significantly greater parasite transmission. The guppies in both experiments were maintained under the same

laboratory conditions and housed in mixed sex shoals at similar sex ratios prior to use. As all fish within a single experimental shoal were taken from the same stock tank, not all stock females were used in the experiments because of the originally higher female:male ratio. The two studies were carried out 12 months apart, but at a similar time of year, under the same temperature, lighting and feeding regimes. However, the fish did originate from different stocks, and although they are both ornamental lines, the male guppies used in this experiment (phenotypically similar to the Green Cobra variety) showed considerably more courtship displays than the males used in the previous experiment (a hybrid between Tuxedo and an unidentified ornamental guppy variety; see Richards et al., 2010). The only other difference between the two studies was the lower initial parasite burden in the present experiment (mean \pm SE = 30 ± 14 worms/fish) compared to the previous study (mean \pm SE = 111 ± 12 worms/fish, see Richards et al., 2010). Possibly, parasite burden affects courtship displays, which is consistent with a previous study (Kennedy et al., 1987) that showed that males with a high gyrodactylid parasite burden showed a lower display rate. In both studies, the fish had previous *G. turnbulli* infections (acquired in the pet trade) which had been successfully treated 3–4 months prior to the experimental infections. As guppies display both acquired and innate immunity against gyrodactylids (e.g. Scott and Robinson, 1984; Cable and Oosterhout, 2007a), parasite population growth may have been slightly reduced in the current study by residual acquired immunity but this would not have affected our key findings.

Focal fish in the current study altered their shoaling behaviour when infected. These fish formed tighter shoals and were more frequently observed shoaling compared to uninfected counterparts. This agrees with previous studies that have shown infected fish have an increased tendency to shoal (reviewed in Barber et al., 2000). For example, parasitised and unparasitised banded killifish (*Fundulus diaphanous*) and cave mollies (*Gambusia affinis*) prefer to shoal with uninfected conspecifics (Krause and Godin, 1996; Töbler and Schlupp, 2008). Contrary to Croft et al.,'s (2011) observations on wild female guppies, but in line with our previous findings on ornamental fish (Richards et al., 2010), non-focal individuals did

not alter their shoaling behaviour following introduction of the infected focal fish. This was despite a small proportion of infected fish displaying clamped fins (indicative of secondary pathology, see Cable et al., 2002), causing these individuals to be phenotypically distinct from uninfected shoal mates. As discussed by Croft et al., (2011), differences in selection pressure between the ornamental and wild guppies may explain the results. Furthermore, in certain conditions, healthy fish may approach individuals showing unusual behaviours to gain information on their condition and evaluate them as potential shoal mates (Barber et al., 2000).

Male guppies were 10 times as likely to contract a parasite infection from the infected focal male than females were from the infected focal female. The higher probability of males transferring an infection seen in this study appeared to be due to increased host contact, although slight differences in parasite population growth between males and females may have contributed. Parasite numbers were similar for male and female guppies at the start of the experiment, but the parasite population growth was marginally faster on focal males than on focal females. We have not observed such differences in host susceptibility between the sexes in previous studies (e.g. Cable and Oosterhout, 2007b), but certainly, different fish stocks do vary in their susceptibility (e.g. Bakke et al., 2002; van Oosterhout et al., 2003). Although differences in immunocompetence between stocks can explain differences in parasite population growth between experiments, it does not explain why parasite transfer among males in the present experiment is 10 times higher than between females. Neither does it explain why both studies (i.e. current versus Richards et al., 2010) differ in the gender that transfers most parasites. Rather, we suggest that the lower initial parasite burden (ca. 30 in the current study compared to >100 worms in Richards et al., 2010) may have allowed males to continue their courtship displays in the present experiment. Higher levels of courtship could explain why males in the present experiment showed a higher level of contact (i.e. shoaling parameters). Thus, this study effectively presents the 'other side of the coin' to our previous findings (Richards et al., 2010). Regardless, of whether male (current study) or female (Richards et al., 2010) hosts display greater contact, it is the degree of host contact which is the major factor influencing parasite transmission in shoals of guppies, and probably more generally in fish shoals. This supports the suggestion by Endler (1995) that a shorter nearest neighbour distance of guppies would facilitate inter-host transmission of gyrodactylids, and that in social hosts like guppies, the frequency of social contact regulates disease epidemics rather than host density (Johnson et al., 2011). Overall, this study adds to the growing body of evidence showing the importance of host behaviour in controlling parasite transmission within a group-living host species.

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