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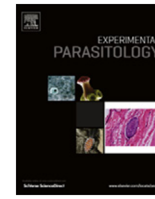

Interactions between males guppies facilitates the transmission of the monogenean ectoparasite *Gyrodactylus turnbulli*

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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.

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 biassed (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

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aim of the current study is to assess 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 ± 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 ± 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