Animal Behaviour 107 (2015) 253–261

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Experimental infection with the directly transmitted parasite *Gyrodactylus* influences shoaling behaviour in sticklebacks

Anna K. Rahn*, Daniela A. Hammer, Theo C. M. Bakker

Institute for Evolutionary Biology and Ecology, University of Bonn, Bonn, Germany

A R T I C L E I N F O

Article history:
Received 26 January 2015
Initial acceptance 20 April 2015
Final acceptance 18 June 2015
Available online 1 August 2015
MS. number: 15-00066R

Keywords:
disease transmission
fish
Gasterosteus aculeatus
grouping
host–parasite interaction
parasitism
social behaviour

Animals usually benefit from joining groups, but joining a group can also come at a cost when members expose themselves to competition and the risk of contracting a contagious disease. Therefore, individuals are expected to adjust grouping behaviour to the ecological circumstances, their own competitiveness and the composition of the group. Here, we used experimental infections and classic binary choice tests to test whether the monogenean flatworm *Gyrodactylus* spp. has the potential to influence shoaling behaviour in the three-spined stickleback, *Gasterosteus aculeatus*, a model organism in behavioural ecology and evolutionary biology. *Gyrodactylus* spp. is a genus of widespread and rather inconspicuous, small (<0.5 mm) ectoparasites on fishes with the ability to cause severe damage to its host. *Gyrodactylus* species infecting sticklebacks have short generation times and those species typically residing on the skin or fins of their hosts are easily spread via body contact. In our experiments uninfected sticklebacks significantly preferred a group of uninfected fish over a group of *Gyrodactylus*-infected fish, while *Gyrodactylus*-infected sticklebacks did not discriminate between the two stimulus shoals with regard to their *Gyrodactylus* infection status. As infected fish were in poorer condition, were less likely to shoal and had a relatively heavy spleen, we suggest a generally reduced health state caused by the infection as a possible indirect mechanism of the altered shoaling preference. Although parasitism has been shown to play an important role in group formation, only a few studies have used experimental infections to directly test its influence on shoaling decisions. Our results show that *Gyrodactylus* spp. can influence shoaling decisions in three-spined sticklebacks and affirm the suitability of the *Gyrodactylus*–stickleback system for studying the role of parasitic infections on host group dynamics.

Forming groups is a widespread phenomenon in animals: associations range from temporary loose aggregations of individuals to eusociality known from hymenopterans, termites and mole-rats (Alexander, 1974). Generally, reduced predation risk (Hamilton, 1971) and more efficient foraging (Clark & Mangel, 1986; Pitcher & Parrish, 1993) are considered the main advantages of being a member of a social group. On the other hand, by joining a group, individuals expose themselves to competition and often increase their risk of contracting a contagious disease. Thus, an individual should adjust its decision to join a certain group not only to the ecological conditions and to the composition of the group with regard to body size, morphology and kinship, but also to its own competitiveness (see e.g. Krause & Ruxton, 2002 for a review). Parasites (referring to macroparasites in this article) play an important role in this context. By definition, parasites cause harm to their host. By impairing certain physical abilities, generally weakening their host, or by changing the appearance of their host, parasites can reduce their host's competitiveness and make it conspicuous. Effects of parasites on their host can ultimately lead to altered group composition if conspecifics are able to identify infected individuals and/or infection affects an individual's tendency to join a group (Krause & Ruxton, 2002; Loehle, 1995).

The detrimental effects that parasites have on their host can vary from hardly noticeable use of resources tolerated by an otherwise healthy host to conspicuous coloration (e.g. visible spots caused by trematodes underneath the transparent skin of fish hosts or in the eye stalks of snails), changes in behaviour (Moore, 2002), host castration or even death. Therefore, the nature of the parasitic infection, in terms of the parasite's virulence, site of infestation, life cycle and mode of transmission (Côté & Poulin, 1995), determines how the social behaviour of a host species can influence the dynamics of a parasitic infection and vice versa. Among parasites with a simple life cycle two different types can be distinguished: mobile

* Correspondence: A. K. Rahn, Institute for Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, 53121 Bonn, Germany.
E-mail address: arahn@evolution.uni-bonn.de (A. K. Rahn).

http://dx.doi.org/10.1016/j.anbehav.2015.07.004
0003-3472/

parasites, such as biting flies on feral horses and *Argulus* spp., a crustacean sucking blood from sticklebacks (Poulin & FitzGerald, 1989; Rubenstein & Hohmann, 1989; Rutberg, 1987), that actively seek new hosts and whose intensity of infection decreases with increasing host group size, and parasites that increase in number when their hosts form larger groups (Côté & Poulin, 1995; Krause & Ruxton, 2002). In terms of their influence on host grouping, parasites in the second category resemble contagious diseases typically caused by microparasites. Their establishment in a group of hosts typically lacks a dilution effect and transmission success often increases in denser host groups as was observed, for example, for intestinal worms in feral horses (Rubenstein & Hohmann, 1989) or for viviparous gyrodactylids on fish (Boeger, Kritsky, Pie, & Engers, 2005; Johnson, Lafferty, van Oosterhout, & Cable, 2011).

A large body of data on social behaviour and its interaction with parasitic infections has been gathered by studying different fish species, predominantly those living in freshwater habitats (see Barber, Hoare, & Krause, 2000 for a review). Here, we look at the possible impact of *Gyrodactylus* spp. on the shoaling decisions of three-spined sticklebacks, *Gasterosteus aculeatus*. *Gyrodactylus* spp. is a widespread and rather inconspicuous ectoparasite on fishes (fresh and salt water, see Bakke, Cable, & Harris, 2007 for a review). The monoxenous (one host life cycle) parasite is directly transmitted via body contact between hosts. Viviparous *Gyrodactylus* species, such as those infecting sticklebacks, give birth to a fully developed embryo that already contains a second embryo. Owing to this special mode of reproduction and the direct transmission via body contact, single worms can initiate an epidemic which is why parasitologists often refer to *Gyrodactylus* as a microparasite. Still, to avoid confusion with conventions established among biologists that allocate parasites to the terms micro- and macroparasite based on their size, in this paper we refer to *Gyrodactylus* spp. as a macroparasite. Some *Gyrodactylus* species have been shown to cause severe damage to their specific host, *Gyrodactylus salaris* on wild and farmed salmon in Norway being the most prominent example due to severe losses in fish stocks since the 1970s (Bakke et al., 2007). Pathogenicity in this genus is strongly dependent on the *Gyrodactylus* species (see e.g. Cable & van Oosterhout, 2007). Most studies on the interaction of *Gyrodactylus* and shoaling behaviour of its fish host have been done on guppies and mainly on the *Poecilia reticulata*–*Gyrodactylus turnbulli* system. In guppies, *G. turnbulli* causes abnormal swimming behaviour and clamped fins, both clearly visible symptoms, before infected fish die (Cable, Scott, Tinsley, & Harris, 2002). Female guppies usually shoal more than males and transmission of *Gyrodactylus* is more easily facilitated among interacting conspecifics (Richards, van Oosterhout, & Cable, 2010; Stephenson, van Oosterhout, Mohammed, & Cable, 2015; but see Richards, van Oosterhout, & Cable, 2012). Experimental infection showed a negative effect on shoal cohesion in studies by Croft et al. (2011), and Hockley, Wilson, Graham, and Cable (2014), but Richards et al. (2012), working on the same species, but a different stock, found infected guppies formed even tighter shoals than uninfected guppies. To our knowledge, whether individual guppies (or any known host for *Gyrodactylus*) would discriminate infected from uninfected conspecifics in shoal choice decisions has never been tested directly. For our experiments, we chose the three-spined stickleback. Sticklebacks are a widely distributed host for *Gyrodactylus* (see e.g. Kalbe, Wegner, & Reusch, 2002; Malmberg, 1970; Özer, Öztürk, & Öztürk, 2004; de Roij & MacColl, 2012; Sulgostowska & Vojtkova, 2005) and their shoaling behaviour has been well studied (see e.g. Frommen, Hiermes, & Bakker, 2009 and citations therein), which makes this species particularly interesting for studies on the impact of parasites on host–host interactions. Sticklebacks form loose shoals during their nonreproductive phase (Wootton, 1976) and their shoaling decisions are known to be influenced by group composition, for example with regard to body size (Hoare, Krause, Peuhkuri, & Godin, 2000), as well as by the nutritional state of the choosing individual (Frommen, Luz, & Bakker, 2007). Parasites have also been recognized as a factor interfering with shoaling behaviour in sticklebacks. In shoal choice tests, uninfected sticklebacks significantly preferred shoals of uninfected conspecifics over shoals containing individuals infected with either the ectoparasitic copepod *Argulus canadensis* (see Dugatkin, FitzGerald, & Lavoie, 1994), *Schistocephalus solidus* (see Barber, Downey, & Braithwaite, 1998) or *Glugea anomala* (see Ward, Duff, Krause, & Barber, 2005). In contrast to *Gyrodactylus* spp., these parasites cause clearly visible signs of infection such as a swollen abdomen (*S. solidus*) or white cysts several millimetres in diameter (*G. anomala*), or are conspicuous themselves because of their body size (*A. canadensis*). A possible impact of *Gyrodactylus* spp. on the behaviour of sticklebacks has not been tested. Compared with guppies or salmonids, consequences of infection are usually not as severe in sticklebacks (see e.g. Konijnendijk, Raeymaekers, Vandeuren, Jacquemin, & Volckaert, 2013; Lester, 1972; de Roij, Harris, & MacColl, 2010) and low infestations are usually assumed to be tolerated by an otherwise healthy host. Dynamics of *Gyrodactylus* infections can be complex due to the parasite's mode of reproduction and because hosts differ in their susceptibility. On a newly infected stickleback responding to the infection the worm population often first increases before the highest level of infection is reached and the population declines again until the infection is eliminated (Bakke et al., 2007; de Roij et al., 2010). Still, *Gyrodactylus* spp. infecting three-spined sticklebacks cause immune reactions in their host (Lester, 1972) and increase mortality (Lester & Adams, 1974). Therefore, uninfected fish would clearly benefit from avoiding infected conspecifics if this reduces infection risk.

In this study, we tested whether three-spined sticklebacks are able to distinguish between *Gyrodactylus*-infected or uninfected conspecific shoals, and if so, whether their shoal choice is influenced by their own *Gyrodactylus* infection status. We used experimentally infected sticklebacks and quantified shoaling preferences in binary shoal choice tests. We hypothesized that, given that sticklebacks are able to distinguish between infected and uninfected conspecifics, uninfected individuals would avoid contact with infected fish. For infected fish the situation is not that clear. On the one hand, individuals already struggling with an infection should avoid increasing their parasite load and the potential costs associated with it. On the other hand, infection may be demanding in terms of energetic expenditure and reduce an individual's competitiveness. In this case it could pay an individual to shoal with weak(er) competitors. Indeed, a preference for poor competitors has been found in minnows, *Phoxinus phoxinus* (Metcalfe & Thomson, 1995). Thus, we expected infected individuals not to show a clear preference for one of the shoals.

METHODS

Origin, Disinfection and Maintenance of Fish

Adult male and female three-spined sticklebacks were caught from a freshwater pond situated in the backyard of the Institute for Evolutionary Biology and Ecology (50°44′ N, 7°4′ E; Bonn, Germany) where all experiments took place. Sticklebacks in that pond show naturally occurring *Gyrodactylus* spp. infections. For the shoal choice experiments approximately 230–300 fish were caught in March and between June and October 2010 using minnow traps and were carried in buckets to the building (distance < 40 m). Sticklebacks not showing any sign of reproductive activity were disinfected by placing them in a 0.015% formalin solution for 40 min. Formalin is commonly used against ectoparasites on fish