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ORIGINAL ARTICLE

Specific manipulation or systemic impairment? Behavioural changes of three-spined sticklebacks (*Gasterosteus aculeatus*) infected with the tapeworm *Schistocephalus solidus*

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Abstract
Manipulation of host behaviour by parasites to enhance transmission to the next host is a fascinating yet controversial phenomenon. This is because it is often hard to discriminate specific manipulation from unspecific side effects of the infection, i.e. a systemic impairment that could be due to a weakened general body condition. When infected with the tapeworm *Schistocephalus solidus*, stickleback fish swim closer to the water surface and exhibit reduced predation avoidance behaviour, which facilitates transmission of the tapeworm to the final host, most often a fish-eating bird. We here tested whether the behavioural changes of infected sticklebacks are specific to contexts where they would indeed enhance transmission, or rather more general. Therefore, we compared the behaviour of sticklebacks that were experimentally infected with *S. solidus* or left uninfected, in settings where the behaviour would influence parasite transmission to a high degree (response to a bird predator stimulus) or to a lesser extent (exploration of a new environment, activity while foraging). As expected, infected sticklebacks returned much faster to foraging after the bird predator stimulus and spent more time close to the water surface, compared to non-infected sticklebacks. By contrast, exploration of a new environment and activity while foraging did not differ between infected and non-infected sticklebacks. This suggests that alteration of the sticklebacks' behaviour when infected with *S. solidus* is indeed due to specific manipulation of the predator avoidance behaviour and not a general, systemic impairment of infected sticklebacks.

Significance statement
Manipulation of host behaviour by parasites is a fascinating but controversial phenomenon, since it is often difficult to disentangle if they are specifically induced or just a side effect of the infection. Stickleback fish, when infected with a tapeworm, change their behaviour dramatically; they swim closer to the water surface and reduce their escape behaviour, which exposes them to predation by birds, the final hosts of the parasite. We observed that sticklebacks infected with the tapeworm perform equally well as non-infected conspecifics in contexts with low relevance for parasite transmission, such as exploration of a new environment and foraging activity. However, the same infected sticklebacks exhibited the expected reduced escape behaviour when tested with a simulated bird attack. Our study suggests that the parasite specifically induces the sticklebacks' behavioural changes and does not simply cause a systemic impairment.

Keywords Stickleback · *Gasterosteus aculeatus* · *Schistocephalus solidus* · Behaviour · Host manipulation · Host-parasite interaction · Systemic impairment

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Introduction

Many parasites with complex life cycles, i.e. where growth and reproduction take place in different host species,

manipulate the behaviour of their hosts to increase the likelihood of transmission from one host to another (Barber et al. 2000; Cézilly et al. 2010; Lafferty and Shaw 2013; Hebert and Aubin-Horth 2014; Poulin and Maure 2015). The underlying mechanisms are often complex and difficult to disentangle (Lafferty and Shaw 2013), and it is not always clear if the behavioural changes are specifically induced by the parasites or rather side effects of the hosts' response to infection (Poulin 1995; Lefevre et al. 2009).

With the present study, we test the specificity of parasite-induced behavioural changes by comparing behaviours of infected and non-infected hosts in contexts, with high to little relevance for the transmission of the parasites to the next host. If behavioural changes occur across different contexts, they are likely due to a systemic impairment of the host. If they occur predominantly in contexts relevant to parasite transmission, it is more likely that they are specifically induced by the parasite.

The cestode *Schistocephalus solidus* is such a trophically transmitted parasite and has become an important model to investigate behavioural changes of its hosts upon infection. *S. solidus* has a three-host life cycle, with cyclopoid copepods as first, three-spined sticklebacks (*Gasterosteus aculeatus*) as specific and obligatory second and fish-eating birds as final hosts (Clarke 1954). *S. solidus* induces behavioural changes in both its intermediate hosts, which in both cases facilitate the parasite's transmission to the next host (Giles 1983; Milinski 1985; Barber et al. 2004; Hammerschmidt et al. 2009; Parker et al. 2009; Weinreich et al. 2013; Hafer and Milinski 2016a, b). In the copepod, *S. solidus* reduces the swimming activity of the host during the first weeks of infection, when the parasite is still unable to infect the stickleback host, thus reducing the likelihood of being spied by a predator (Weinreich et al. 2013). Later, when the parasite becomes infective for sticklebacks, higher swimming activity of the copepod is triggered by *S. solidus*, making it more prone to be detected by predators, such as the three-spined stickleback (Franz and Kurtz 2002; Hammerschmidt et al. 2009; Parker et al. 2009).

In the stickleback, *S. solidus* grows in the body cavity and starts to change host behaviour when it has reached 50 mg, the threshold weight for reproduction in the gut of the final bird host (Tierney and Crompton 1992; Barber et al. 2004). *S. solidus*-infected sticklebacks become bolder under the risk of predation (Milinski 1985), are more often found separate from shoals of conspecifics (Barber and Huntingford 1995) and show reduced predation avoidance behaviour after an artificial bird strike (Barber et al. 2004). In addition, *S. solidus*-infected sticklebacks swim more often and longer close to the water surface (Giles 1987; Lobue and Bell 1993; Quinn et al. 2012). The infection can further cause demelanization of the skin leading to a brighter appearance of infected sticklebacks (Lobue and Bell 1993; Ness and Foster 1999). In concert, these *S. solidus*-induced changes make their stickleback hosts much more prone to predation by the final bird host.

Although the behavioural alterations of sticklebacks by *S. solidus* are well described, it is still not clear if the cestode specifically induces the changes in host behaviour or if they are a side effect of a systemic impairment. The phenotypic alterations of sticklebacks by the parasite are enormous, the parasites can grow up to half of the hosts' body weight (Arme and Owen 1964) and the swelling of the abdomen can cause the head and tail of the hosts to bend upwards (Arme and Owen 1967). At the same time, the growing parasite constricts the intestine of the stickleback and thereby restricts the meal size which leads to extended feeding activity (Barber and Huntingford 1995), whereby smaller and energetically more valuable food items are preferred (Milinski 1984). *S. solidus*-infected sticklebacks show impaired development of their gonads (Bagamian et al. 2004; Schultz et al. 2006; Heins et al. 2010) and reduced reproductive activity, which varies in severity across host-parasite populations (Macnab et al. 2009; Heins and Baker 2014). Even though these phenotypic alterations of the stickleback host by the parasite are prominent, they do not appear to be directly causative for the hosts' behavioural changes.

A study on neurotransmitters in the brain of *S. solidus*-infected sticklebacks revealed a pattern consistent with chronic stress (Øverli et al. 2001) but did not provide evidence for a more specific neuronal manipulation by the parasite. However, the sticklebacks' behaviour might also be influenced indirectly by *S. solidus*. It was suggested that in sequential *S. solidus* infections, host manipulation by the first parasite must be sabotaged by a second, younger parasite, which was not yet infective for the final host, but this was not observed and it was concluded that host behavioural manipulation was rather an unspecific side effect of the infection (Hafer and Milinski 2016b). Furthermore, a specific manipulation must be independent of the hunger status of infected sticklebacks, but starvation and satiation influenced the risk averseness of infected and uninfected sticklebacks in a similar manner and it was suggested that increased energy drain dominates behavioural changes during *S. solidus* infection (Hafer and Milinski 2016b). Both experiments support the idea that *S. solidus*-induced host behavioural changes are unspecific side effects of the infection but do not formally prove this assumption.

The high energy demand of the growing *S. solidus* increases the respiration activity of infected sticklebacks (Meakins and Walkey 1975). Accordingly, infected sticklebacks have higher oxygen demands, which may stimulate their swimming close to the water surface, with higher accessibility of atmospheric oxygen (Lester 1971; Giles 1987). Lobue and Bell (1993) suggested that *S. solidus* might alter the buoyancy of its host and tested the floating capacity of anesthetized infected and non-infected sticklebacks in solutions with increasing salinity. Indeed, *S. solidus*-infected sticklebacks were more buoyant, but the density of the cestode, when dissected from the host, was higher than that of the stickleback (Lobue and Bell 1993). This means that the hosts

must have used their swim bladders or other upwelling forces to reach higher buoyancy despite the heavier parasite.

Ness and Foster (1999) stated that the *S. solidus* infection makes sticklebacks less capable of performing evasive manoeuvres and leads to struggling and slow locomotion. The overall decreased host mobility was also interpreted as an incidental side effect of physical alterations (Lobue and Bell 1993). The swollen abdomen as well as the increased need of energy and oxygen could induce a decrease of escape behaviour by impeding the swimming abilities of the fish. Hence, these changes could be a side effect of the infection, which could also be considered a type of host manipulation (Cézilly et al. 2010), but unspecific (Poulin 1995). One of the earlier studies noted that infected sticklebacks could still do rapid swimming moves when startled, while also describing deficits of the stamina of infected fish (Arme and Owen 1967).

Taking these studies together, a fundamental question remains unanswered: are the observed alterations of the behaviour of infected stickleback merely the result of physical and physiological changes causing systemic impairment or are they the consequence of a specific manipulation by *S. solidus*? We hypothesize that if the changes in behaviour of infected sticklebacks were unspecific and due to the massive phenotypic alterations creating systemic impairment, infected sticklebacks should be affected in various behavioural contexts, most evidently in those which require heavy duty physical activity. Consequently, infected sticklebacks should also differ in locomotion, foraging and exploration behaviours from non-infected sticklebacks in contexts with minor importance for the parasites' transmission success. From an evolutionary perspective, a general impairment of the host's ability to swim, e.g. during foraging, would be disadvantageous for the developing *S. solidus*. For exploration behaviour, a similar rationale applies: the host exploring its environment should be beneficial for the parasite, if it leads to new food sources; therefore, the parasite should not alter such behavioural traits, or if it does, it should do so in a manner that facilitates its transmission.

We therefore compared the activity of *S. solidus*-infected and non-infected sticklebacks in three different scenarios. (1) We analysed the ability of infected and non-infected sticklebacks to follow a moving food bait (Fig. 1b). If *S. solidus* caused a systemic impairment, we assumed that infected sticklebacks would be less capable of performing this task. (2) The sticklebacks were exposed to an exploration of a new environment setting, adapted from Dingemanse et al. (2007), and their behaviours were analysed (Fig. 1b). We expected to see differences in the exploration behaviour between infected and non-infected sticklebacks, if *S. solidus* would cause a systemic physical impairment. (3) It was previously established that *S. solidus*-infected sticklebacks perform bolder than non-infected ones (Milinski 1985; Barber et al. 2000; Barber and Svensson 2003) and increased boldness of *S. solidus*-infected sticklebacks during foraging had to be expected (Milinski 1984). Therefore, we analysed the sticklebacks' foraging activity after an artificial bird strike (Barber et al. 2004) as a control for the parasite-induced changes of host behaviour.

Materials and methods

Sticklebacks

Experimental sticklebacks and *S. solidus* were laboratory-bred offspring (F1) of parental sticklebacks and parasites caught in a brook (Ibbenbürener Aa, 52° 17′ 31.76″ N, 7° 36′ 46.49″ E) in northwest Germany. After initial feeding with *Artemia* naupliae in 0.5-L cups, sticklebacks were kept in 14-L tanks in recirculated tap water at a 16/8 h light/dark cycle at 16 °C and fed daily ad libitum with blood worms (Chironomidae). Prior to the experiment, sticklebacks from five families were pooled and subjected to a DNA-based sex determination assay as described by Peichel et al. (2004) and individually tagged with visible implant elastomer tags (Northwest Marine Technology Inc., USA) (Henrich et al. 2014). For a timeline of the experimental steps, see Fig. 1c. For the present study, 44 adult sticklebacks (14 months old) from the family pool were distributed in groups of 11 individuals to four 14-L tanks, subsequently named 'home tanks' in a gender-balanced design. The distribution of infection treatments and sex across the four home tanks is illustrated in Table 1.

S. solidus parasites

Fully developed parasites were collected from wild-caught sticklebacks (Ibbenbürener Aa; see above) and bred in an in vitro culture (Smyth 1946; Wedekind 1997; Luescher and Wedekind 2002). *S. solidus* eggs were washed with sterilized tap water and stored at 4 °C in the dark until use. For the infection experiment, *S. solidus* eggs were transferred to 20 °C for 3 weeks and hatching was induced by exposure to light. Single coracidia, from five *S. solidus* families, were transferred to individual wells of 24-well plates with a single copepod (*Macrocyclops albidus* from a laboratory culture; see van der Veen and Kurtz 2002) per well in 1 mL tap water. The infection status of the copepods was determined microscopically 2 weeks post exposure. Sticklebacks were starved for 2 days and transferred to individual jars with 500 mL tank water, acclimatized overnight and exposed to single copepods infected or not infected (sham) with *S. solidus*. On the next day, the water from the exposure tanks was sieved to confirm ingestion of the copepods and the sticklebacks were returned to their home tanks. From previous infection experiments, an infection success of *S. solidus* of about 50% was expected for this population (JPS, personal observation, but see also Scharsack et al. 2007). Sticklebacks were screened for the

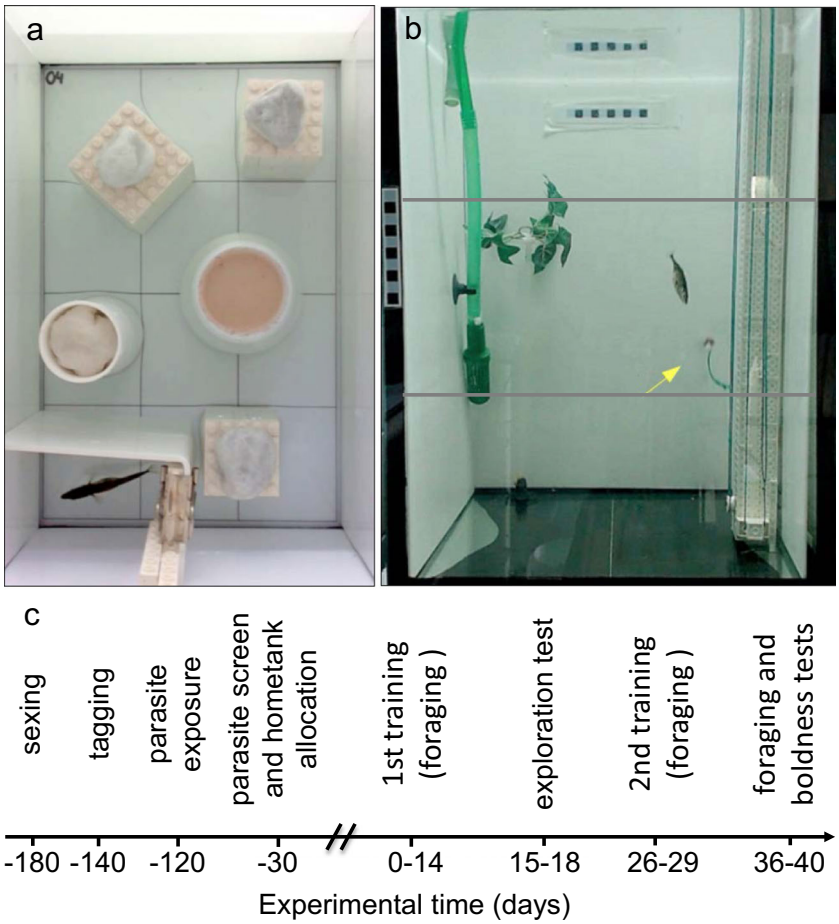

Fig. 1 Experimental tanks and timeline. **a** Exploration of a new environment. Five obstacles emerged from 5 cm of tank water. A removable acclimation chamber was located in the left corner. **b** Foraging. The food bait (*arrow*) was pulled from the surface to 50 cm depth and back up again with a speed of 11–12 rounds/min (*black*/*grey scale* = 10 cm). **c** Timeline of main experimental steps

presence of *S. solidus* plerocercoids 90 days after parasite exposure by visual inspection with a binocular. Infection was confirmed for fish with swollen abdomens, when parasite movements were observed under the abdominal skin. Accordingly, the group of *S. solidus*-exposed sticklebacks split up into exposed and infected (inf) and exposed but uninfected (exp not inf) (Table 1) (for infection time, see Fig. 1c).

Exploration of new environment test

Here, 11 identical tanks were used in parallel to assess the exploration behaviour of the sticklebacks from one home tank contemporarily. Each test tank (20 × 30 × 20 cm^3) was equipped with a camera and five objects with an approximate size between 4.5 × 4.5 × 7.0 cm^3 and 7.5 × 7.5 × 7.5 cm^3 and a grid of 7 cm × 6 cm on the ground (Fig. 1a). The test tanks were filled with 5 cm of water only, so that the sticklebacks could not swim above the objects. Each tank was lined with opaque white blinds, and all tanks were further isolated from the experimenter by white curtains to avoid visual irritation of the fish.

On the day of the test (Fig. 1c), all sticklebacks from one home tank were transferred to the exploration test tanks. The exploration of a new environment tests were conducted between 10 and 19 h. The sticklebacks were allowed to acclimate to the test tanks for 300 s inside a separated area of

Table 1 Home tank composition with respect to gender and infection treatment

Home tank	Sham-exposed control		Exposed not infected		Exposed infected	
	Female	Male	Female	Male	Female	Male
1	2	2	2	2	2	1
2	2	2	2	0	2	3
3	2	1	1	2	2	3
4	2	2	1	2	2	2

9×6 cm^2 in a corner of the tank (Fig. 1a). After acclimation, the separation walls were gently removed and the sticklebacks released to explore the new environment for an additional 300 s, during which their activity was recorded with the camera. After the test, the sticklebacks were returned to their home tank and the water in the test tanks was exchanged for the next home tank group. For the exploration of a new environment test analysis, one video recording was blurred and not usable; accordingly 43 of the 44 test individuals were included in the analysis.

Foraging activity

The test tank (40 × 40 × 60 cm) was equipped with opaque blinds on three walls and the front wall was left transparent for observations (Fig. 1b). The tank was filled up to 50 cm with a 1/1 mix of tank and aged tap water. An automatic pulley for a food bait, spanning the entire height of the tank, was used to stimulate foraging activity. The sticklebacks were trained to acclimate to the tank conditions to avoid effects by being exposed to a novel environment in this experiment (for the timeline of the whole experiment, see Fig. 1c). For the first training, home tank groups were transferred as a whole to the foraging test tank and fed ad libitum to train them to expect food therein. Additionally, we performed a second training for the foraging tests with the pulley with each stickleback individually. For the video analysis, the test tank was subdivided into three equal vertical zones (Fig. 1b).

The foraging and boldness tests were conducted between 8 h 30 and 16 h 30. For the foraging test, a bait of approximately 1.2 g of chironomid larvae wrapped in gauze was attached to the pulley (Fig. 1b). The gauze allowed the fish to smell and spot the food but prevented the single larvae from dropping out or being eaten easily. To ensure a high motivation for foraging activity, the sticklebacks were starved for 2 days before this test. For the test, individual sticklebacks were taken from the home tanks in a random order and left in the test tank for 300 s to acclimate. After acclimation, the food bait was moved into the water and up and down in the water column with the automatic pulley to challenge the swimming abilities of the sticklebacks. The bait was pulled for 120 s with a speed of 0.2 ± 0.05 m/s (average ± standard deviation). Then, the bait was removed and the fish was left alone for 300 s.

Boldness test

The boldness test was performed after the foraging test in the same tank. For the test, a fake bird beak was attached to the tank, invisible for the fish while not in use. The food bait from the previous test was placed at the water surface, and when the stickleback approached the bait within one body length, the fake bird beak was triggered and quickly pinched the water surface. Then, the latency to return to the food bait after the bird strike was recorded. Recording was stopped when the stickleback returned to the food bait or at a maximum of 300 s after the simulated bird attack (if the stickleback did not return to the bait).

Acquisition of behavioural data

All behavioural experiments were continuously recorded on digital video (Logitech C910 and C920 web cameras, Logitech, Apples, Switzerland). We used the ANY-maze® tracking software (Version 4.99, Stoelting, Wood Dale, IL, USA) to determine movement parameters such as the position of the fish, number of zone changes (line crossings), its maximum speed, total distance covered and mobile time (Table 2). The videos were analysed automatically with ANY-maze® by tracking the movements of the centre point of the stickleback. For the mobility tracking, a sensitivity of 65% was set in the software. An immobile state was recorded after 2000 ms of the fish not being mobile. To analyse the vertical preferences during the foraging test (Fig. 1b), we subtracted the time spent in the lower third from the time spent in the upper third for each individual stickleback.

It was not possible to analyse all data blind, since the *S. solidus*-infected sticklebacks were obvious due to the swelling of their bodies. However, for uninfected sticklebacks, the observer did not know during the video analysis if they were sham-exposed controls or exposed but not infected.

Statistics

Statistical analyses were performed with R 3.1.2 (R Development Core Team 2015). We visually assessed descriptive statistics (histograms, boxplots, qq-plots) using the car and MASS packages. Our main objective was to compare the three infection treatments, exposed infected (inf), exposed uninfected (exp not inf) and sham-exposed (sham) control sticklebacks. We found that the home tank was an imbalanced factor and suspected that the shared environment of a particular home tank might influence the individual behaviours on display. Since the sticklebacks were adults by the start of the observations, gender was included in the statistics. Thus, we selected the random structure a priori, i.e. controlling for imbalanced factor-level combinations like home tank and gender. We employed an adaptation of a protocol suggested by Zuur et al. (2013). We specified five overall models (Table 3) with the lmer function in the lme4 R packages (Bates et al. 2015). We compared these five models for every single behavioural readout variable (Table 2) and investigated with t and F statistics the contributions of variables in the best-fit model. Models were fitted with maximum likelihood. We assumed an alpha level of 0.05 for fixed effects and random effects; both kinds of effects were reduced stepwise using likelihood ratio tests. We computed hierarchical model testing and summary statistics by subsequently looping over the model fits

Table 2 Behavioural variables recorded during the tests

Variable	Description
Distance	Entire distance in metres travelled during the test
Time mobile	Time in seconds the stickleback was mobile during the test
Line crossings	Number of line crossings between tank partitions (Fig. 1)
Maximum speed	Highest speed (m/s) the stickleback exhibited during the test
Latency to return to food (boldness test only)	Time in seconds until the stickleback returned to the food bait after an artificial bird strike

to compute delta AICc and Akaike weights, implementing model evaluation functions inherent to the R packages MuMIn v1.1 and plyr 1.8. Taking into account the complexity of a model in relation to the approximated log likelihood and number of samples, the best-fit model was selected by the Akaike information criterion for small sample size (AICc), which avoids over-parametrization. Although the mixed model is robust against violations, it should be noted that the sample sizes were small and in some cases over-dispersion and violations of homoscedascity could not be avoided.

Ranking of statistical models

For every behavioural read out variable, the input models m0–m4 (Table 3) were ranked according to the corrected Akaike information criterion (AIC) to account for small sample sizes. The complete model rankings are listed in the supplemental material (Suppl. 1–3). In the following, we present the resulting best-fit models. Since the absence of significant differences between infection treatments was an important result of the present study, also best-fitted models of non-significant results are described in 'Results'.

Results

Exploration

Infected and non-infected sticklebacks did not differ significantly in the majority of measurements taken during the exploration of a new environment test (e.g. 'distance covered', Fig. 2b, Table 4, supplement Table S1). The exception was the

Table 3 Model composition used for maximum likelihood fitting

Model	Formula
m0	Behaviour ~ infection status * sex + (1 | home tank)
m1	Behaviour ~ infection status * home tank + (1 | sex)
m2	Behaviour ~ infection status + (1 | sex)
m3	Behaviour ~ infection status + (1 | sex) + (1 | home tank)
m4	Behaviour ~ infection status + (1 | home tank)

~ model term; + main effects; * main effects plus interactions; (1 | A) fit random intercepts for A

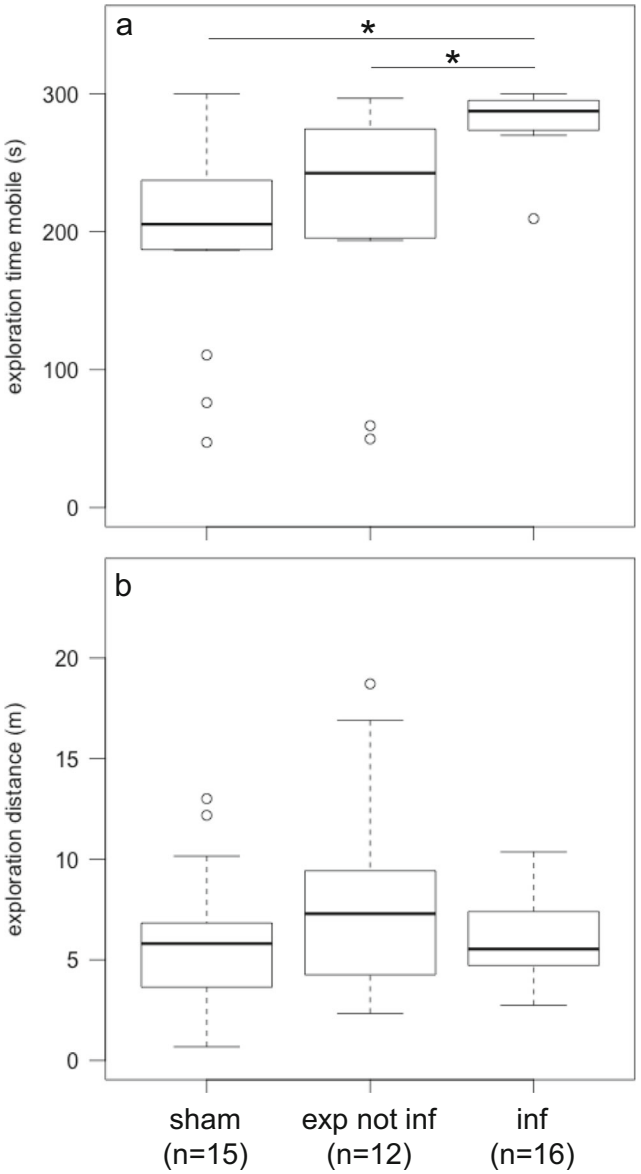

Fig. 2 Exploration of a new environment. **a** Total time the sticklebacks were mobile and **b** the total distance covered within 5 min test duration. Box plots with median, 25th and 75th percentile and upper and lower values which equal or exceed 1.5-fold of the interquartile range (*o* = outliers, *sham* = sham-exposed control, *exp not inf* = exposed but uninfected, *inf* = infected, * $p < 0.05$)

'time mobile' ($F_{1,39.39}$ = 15.177, p = 0.0004), which was higher for infected sticklebacks compared to non-infected conspecifics (Fig. 2a, Table 4). 'Line crossings', 'distance covered' and 'maximum speed' did not differ between infection groups (Table 4). In summary, in the exploration of a new environment test, infected sticklebacks were mobile for a longer duration than non-infected sticklebacks; other than that, the infection treatment groups did not differ significantly from each other.

Foraging activity and vertical preferences

During the foraging test, infected sticklebacks spent significantly more time in the upper third of the tank ($F_{1,22.86}$ = 18.574, p = 0.0003) than their non-infected conspecifics (Fig. 3a). When following the food bait, infected sticklebacks had lower 'maximum speed' ($F_{1,23.95}$ = 14.546, p = 0.0008) than non-infected ones (Fig. 3b). However, infected sticklebacks reached the lower compartment and the number of line crossings between the tank compartments (upper-middle-lower) did not differ between infected and non-infected sticklebacks (Table 4, Suppl. Table S2), indicating that the overall activity was similar for infected and non-infected sticklebacks. This assumption was further supported by the absence of significant differences between infected and non-infected sticklebacks in other activity measurements during the test, such as 'time mobile' and 'distance covered' during foraging (Table 4, Suppl. Fig. S1 a, b, Table S2). In this test, only 26 fish reacted to the moving food bait on the pulley; the remaining 18 did not (it is notable that the composition of either group was different from the boldness test in which also 18 sticklebacks did not participate). However, non-participating sticklebacks in both tests were distributed evenly across infection treatments and gender and did not differ in size from participating sticklebacks (Suppl. Fig. S2, S3 and Table S3, S4).

Boldness

The infection with *S. solidus* had a significant effect on the boldness of sticklebacks following a simulated predator strike ($F_{1,26.00}$ = 16.431, p = 0.0004), and infected sticklebacks (n = 13) took significantly less time to return to the bait than the exposed but uninfected (n = 5) and the sham-exposed control sticklebacks (n = 8) (Fig. 4) (Table 4 and Suppl. Table S1). While some individuals of both non-infected treatment groups did not return to the food bait after the bird strike at all (scoring with a latency of 300 s; see Fig. 4), some of the infected sticklebacks did not exhibit any flight behaviour upon the bird strike and stayed close to the food bait during the simulated attack and consequently scored 0 s to return to the food. From the 44 tested sticklebacks, 18 did not react to the food bait, when it was placed close to the water surface near the artificial bird beak, and the bird strike could not be triggered.

Discussion

Many parasites manipulate their hosts' behaviours, often in a manner that enhances parasite transmission (Adamo and

Table 4 Mixed models. Fixed effects of the best-fit model for each behaviour

Test	Model	Fixed effects	Sum Sq	NumDF	DenDF	*F* value	Pr(>*F*)
Exploration	Distance ~ infection status * sex + (1 | home tank)	Infection status	0.139	1	39.39	0.449	0.5069
		Sex	106.342	1	39.49	3.728	0.0607
		Infection status * sex	5.845	1	39.74	0.564	0.4571
Exploration	Line crossings ~ infection status * sex + (1 | home tank)	Infection status	117.965	1	39.19	0.032	0.8583
		Sex	8373.309	1	39.25	2.112	0.1541
		Infection status * sex	14.607	1	39.38	0.020	0.8870
Exploration	Maximum speed ~ infection status + (1 | sex)[a]	Infection status	0.003	1	42.93	0.791	0.3787
Exploration	time mobile ~ infection status + (1 | home tank)	Infection status	50,931.535	1	39.39	15.177	*0.0004*
Foraging	Distance ~ infection status + (1 | home tank)[a]	Infection status	0.093	1	26.00	0.028	0.8693
Foraging	Line crossings ~ infection status + (1 | home tank)	Infection status	0.268	1	24.78	0.002	0.9656
Foraging	Maximum speed ~ infection status + (1 | sex)	Infection status	0.079	1	23.95	14.546	*0.0008*
Foraging	Time mobile ~ infection status + (1 | home tank)	Infection status	322.274	1	24.79	2.171	0.1532
Foraging	Time upper − lower ~ infection status + (1 | home tank)	Infection status	34,087.136	1	22.86	18.574	*0.0003*
Boldness	Latency ~ infection status + (1 | home tank)[a]	Infection status	134,007.916	1	26.00	16.431	*0.0004*

Significant effects are marked in italics

~ model term; + main effects; * main effects plus interactions; (1 | A) fit random intercepts for A

[a] Models share rank 1 with another model; see Tables S1 and S1

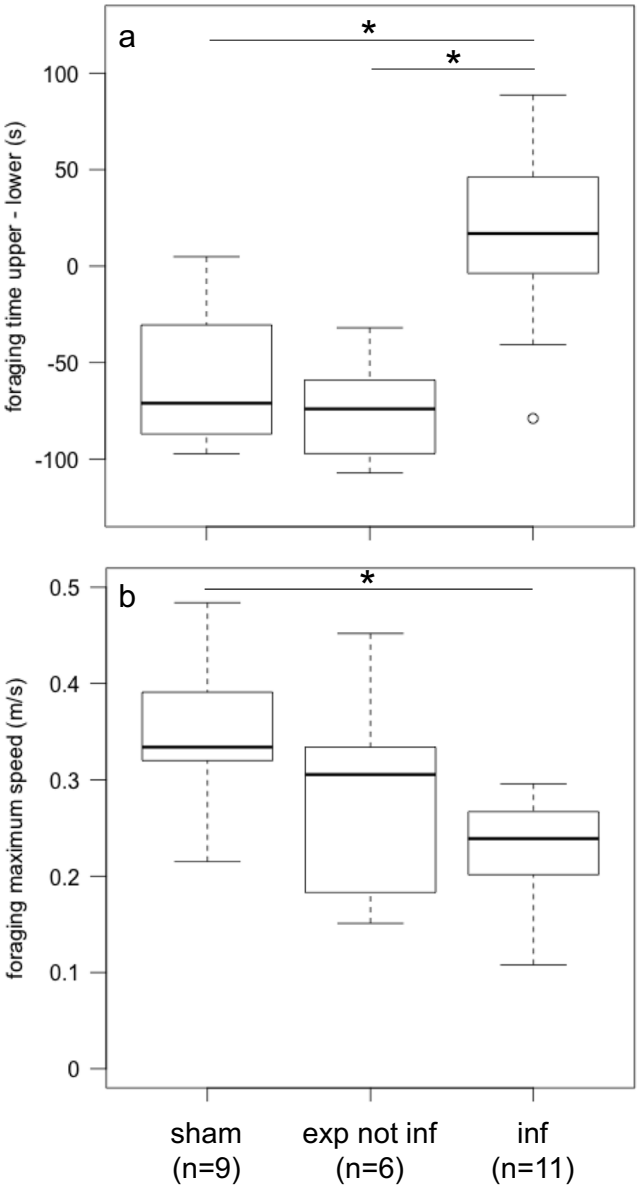

Fig. 3 Foraging activity. **a** Time spent in the upper third minus time spent in the lower third of the tank; **b** maximum speed. Box plots with median, 25th and 75th percentiles and upper and lower values which equal or exceed 1.5-fold of the interquartile range (*o* = outliers, *sham* = sham-exposed control, *exp not inf* = exposed but uninfected, *inf* = infected, * $p < 0.05$)

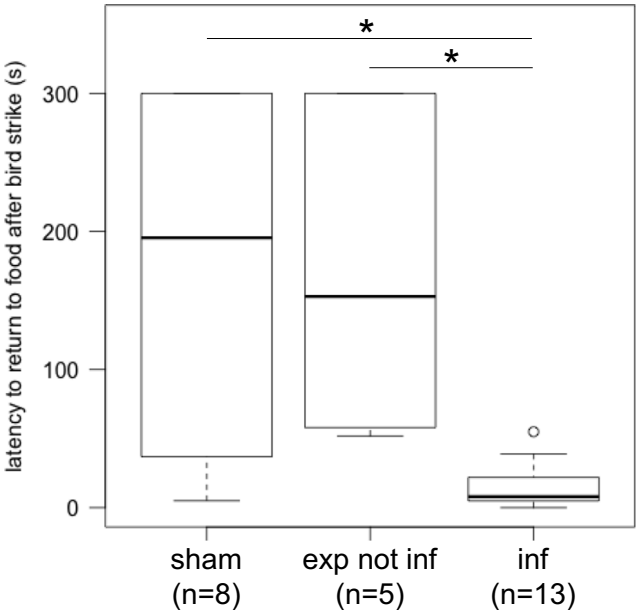

Fig. 4 Boldness test. Latency to return to food bait after simulated bird attack. Only sticklebacks which returned to foraging within 5 min after the bird strike were included. Box plots with median, 25th and 75th percentiles and upper and lower values which equal or exceed 1.5-fold of the interquartile range (*o* = outliers, *sham* = sham-exposed control, *exp not inf* = exposed but uninfected, *inf* = infected, * $p < 0.05$)

Webster 2013). Such behavioural changes of hosts are generally adaptive for the parasite (Lefevre et al. 2009), but it is often difficult to disentangle if the behavioural changes are directly and specifically induced by the parasite (Lafferty and Shaw 2013) or are indirect effects caused by the host's responses to infection (Poulin 1995). We hypothesized that behavioural changes which are induced by unspecific, systemic impairment of the host would be detectable also in situations with low relevance for parasite transmission. Vice versa, specific manipulations would mainly be triggered in contexts which facilitate transmission of the parasite. In three-spined sticklebacks infected with the tapeworm *S. solidus*, behavioural changes are well described and presumably facilitate the transmission of the parasite to its final host (Milinski 1985; Barber et al. 2000). In the present study, differences in behaviours of *S. solidus*-infected and non-infected sticklebacks were observed mainly in contexts relevant for parasite transmission. In contexts with little or no relevance for parasite transmission, differences in behaviours were not detected. Taken together, this suggests that the behavioural changes are specifically induced by *S. solidus* and not simply due to a systemic impairment.

In the foraging test, infected and non-infected sticklebacks did not differ in behaviours which require muscular effort, such as time spent mobile or distance covered; thus, the infection did not cause general physical constraints. In the exploration of a new environment behaviour, infected sticklebacks were similarly active as non-infected sticklebacks, suggesting the absence of a physical or neuronal impairment during exploration in infected fish. Only the time spent mobile was significantly higher in infected sticklebacks. Infected sticklebacks seemed to differ in their quality of movements; they swam continuously at a lower speed, while non-infected sticklebacks showed relatively longer phases of immobility (freezing), followed by faster movements (FS, personal observation). The reflex of freezing, which appears in novel or risky situations as an alternative to flight (Bell 2005), might be repressed by the *S. solidus* infection, and changes in this pattern of movements might again be a specific manipulation by *S. solidus*. The increased time the infected sticklebacks were

mobile could be beneficial for the parasite if this leads to higher detectability by piscivorous birds or to higher likelihood to encounter food sources.

A simulated bird attack (Barber et al. 2004) in combination with food availability (Giles 1983) was used as a control for manipulated behaviour in the present study, and infected sticklebacks returned much faster to foraging after a bird attack than non-infected ones or did not even escape at all. These observations are in line with previous studies, which have observed that *S. solidus*-infected sticklebacks become bolder at risk of predation (Giles 1983; Milinski 1985; Huntingford and Giles 1987). Such behavioural changes only occurred in sticklebacks after the parasites were relatively big (Barber et al. 2004) and had reached the 50-mg threshold for infectivity of the final host (Tierney and Crompton 1992), but not earlier in infection (Aeschlimann et al. 2000). In the present study, the parasites were all at the same age and had become infective for the final host; accordingly, their manipulation of stickleback boldness could be used as a positive control. Potential reasons for variation in the behavioural manipulations, such as differences in parasite weight, were not investigated here. However, in the present study, sticklebacks were starved prior to the tests and infected sticklebacks, nourishing the plerocercoids, might have been in a different hunger state as uninfected ones, which might have influenced their feeding motivation in combination with higher boldness.

In our study, *S. solidus*-infected sticklebacks spent more time in the upper zone of the foraging test tank than non-infected ones. This is another behaviour which was presumably not independent of the parasites' urge to facilitate transmission to the final host. Generally, sticklebacks avoid swimming close to the water surface, since it coincides with a higher risk of being spied by a predator from above, but also from below when being silhouetted against the water surface.

It was observed previously that *S. solidus*-infected sticklebacks swim more often close to the water surface than their non-infected conspecifics (Giles 1987; Lobue and Bell 1993; Quinn et al. 2012). While the increased boldness of infected sticklebacks could be a reason for this behaviour, a physical or physiological reason might be possible, too. It was suggested that hypoxia caused by the parasite burden might stimulate the stickleback hosts to swim in upper water levels with higher accessibility to atmospheric oxygen (Giles 1987). Indeed, Giles (1987) detected higher susceptibility of *S. solidus*-infected sticklebacks to low dissolved oxygen concentrations, but this was also observed with gravid females. However, in the present study, oxygen concentration was even between the upper and lower tank compartments, since the water was circulated and aerated continuously.

In the present study, the increased buoyancy of infected sticklebacks (Lobue and Bell 1993) did not hinder them from reaching the bottom of the tank. But infected fish spent more time in the upper layers which could indicate increased buoyancy while they were still able to make occasional movements to deeper water. However, infected sticklebacks had lower maximum speed in their vertical movements. This might indicate that the parasite also causes physical restrictions to the fish, e.g. by oxygen deprivation or hunger. On the other hand, reduced maximum speed during a flight reaction would facilitate predation and the transmission to the final host.

The present study could use the previously described altered boldness and vertical preferences of *S. solidus*-infected sticklebacks not only as a control but also as hints that the *S. solidus*-induced behavioural manipulations are specific to contexts which are relevant to the parasites' transmission to the final host. Taken together, our findings suggest that behavioural changes that occur in *S. solidus*-infected sticklebacks are specifically induced by the parasite and are not simply due to systemic impairments.

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Compliance with ethical standards

Ethical statement Sticklebacks were maintained and treated in accordance with the EU Directive 2010/63/EU for animal experiments. The present project was approved by an ethical committee of local animal welfare authorities under the project number 87-51.042010A297.

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