Ontogeny of parasite-mediated behaviour change in sticklebacks

Summary

Three spined sticklebacks *Gasterosteus aculeatus* in lacustrine populations are frequently infected with larval stages of the tapeworm *Schistocephalus solidus*, which are only capable of reproduction following the ingestion of their stickleback hosts by fish-eating birds. Earlier studies suggest that these parasites may have evolved mechanisms to manipulate host behaviour in such a way as to maximise the likelihood that infected fish are selectively predated. However, these studies used naturally infected fish, thus precluding a rigorous scientific test of the 'manipulation hypothesis', since it is impossible to determine whether 'odd' behaviour of naturally infected fish is a cause or a consequence of infection.

In this FSBI-funded study, our aim was to identify the temporal changes in the behaviour of experimentally infected sticklebacks, compared with uninfected control fish, and relate this to the developmental stage and growth of the parasite. We exposed 30 lab-bred sticklebacks to controlled doses of *S. solidus* by feeding them experimentally-infected copepods, and used a non-invasive morphometric technique to track the growth of the parasites in the body cavity of infected fish over a 16-week period. Three aspects of fish behaviour were screened every 2 weeks: shelter use, escape response from a model heron, and swimming performance in a miniature 'flume' tank.

Five of the 30 exposed fish developed infections, and plerocercoids grew rapidly, attaining 17-26%% of host weight within 16 weeks. Our results showed that shelter use of experimentally infected fish did not deviate from that of control fish until parasites reached approximately 100mg, but fish harbouring worms over this size showed a marked decrease in the time spent sheltering. The proportion of infected fish reaching cover within 2s after being 'attacked' by a model heron decreased significantly with time since infection. Finally, infection was found to have important consequences for growth and sexual development of sticklebacks. Infected fish (minus the parasites weight) gained weight more slowly than uninfected controls. Experimentally infected females developed lower fat reserves than uninfected controls, but (unexpectedly) had larger ovaries at the end of the study.

Plate 1. *Andreas Svensson (the funded RA) and Katie Woodhouse (undergraduate student) using the digital video camera and image analysis system funded by the FSBI small research grant to examine the swimming behaviour of experimentally infected 3-spined stickleback* Gasterosteus aculeatus

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Introduction

Three spined sticklebacks *Gasterosteus aculeatus* in lacustrine populations are frequently infected with larval stages of the tapeworm *Schistocephalus solidus*, which are only capable of reproduction following the ingestion of their stickleback hosts by fish-eating birds. Earlier studies suggest that these parasites may have evolved mechanisms to manipulate host behaviour in such a way as to maximise the likelihood that infected fish are selectively predated (see Barber *et al* 2000 *Rev. Fish Biol. Fish*. **10**, 131-165). However, these studies used naturally infected fish, thus precluding a rigorous scientific test of the 'manipulation hypothesis', since it is impossible to determine whether 'odd' behaviour of naturally infected fish is a cause or a consequence of infection.

In this study, our aim was to identify the temporal changes in the behaviour of experimentally infected sticklebacks, compared with uninfected control fish, and relate this to the developmental stage and growth of the parasite. In addition, we examined in detail the effects of experimental infection on growth, sexual development and body condition of host sticklebacks.

Methods

Adult male and female sticklebacks were brought into breeding condition and allowed to breed. Forty size-matched juvenile fish (0.13 ± 0.03g) were selected from holding tanks for the experiment. Lab-cultured copepods (*Cyclops strenuus*) were each fed a single *S. solidus* coracidium that had been hatched from incubated eggs recovered from a single adult worm (see Barber et al 2001 *Proc. Roy. Soc. Lond*. **B268**, 71-76). Copepods were screened for infection status after 27d and those harbouring a single infective parasite were fed to 30 of the sticklebacks. The remaining 10 fish (controls) were fed a non-exposed copepod. Infections were carried out under UK Home Office project licence PPL40/2240.

Sticklebacks were housed individually in perforated 25-L aquaria within a filtered recirculating system, maintained at 18 ± 0.5 ºC on an 11L: 13D photoperiod and fed live, lab-reared whiteworms (*Enchytraeus* sp.) at a ration of 8% wet body weight per day. At two-week intervals fish were weighed (to 0.001g) and photographed digitally in dorsal profile. We used image analysis software (ImageTool™) to measure standard length (to 0.01mm) and dorsal profile area (to 0.01mm^2), which allowed parasite weight to be estimated in infected fish (Barber 1997 *J. Fish Biol*. **51**, 654-658).

Shelter use behaviour was screened at weekly intervals. Time-lapse video was used to record the location of fish in their 'home' tanks over a 30-minute period. The escape responses of sticklebacks from a standardised model heron attack was also recorded on digital video every two weeks and analysed frame-by-frame.

After 16 weeks, all fish were sacrificed humanely, weighed, measured and dissected to confirm infection status, identify gender and quantify various body condition parameters (relative liver mass, relative gonad mass, relative parasite weight).

Results

Plerocercoids developed in 5 of the 30 exposed fish. The sample of fish used in the study was (unintentionally) female-biased, and all infected fish were females. Fig 1 shows the typical morphological changes associated with infection compared with a control fish. The morphometric that gave the most consistent and earliest indication of successful infection was the body width at the pectoral fins, which showed significant variation between infection classes from week 6 post-exposure. The length-corrected dorsal profile area of infected fish was also significantly elevated from week 8 post-exposure.

There was no detectable effect of infection status on the trajectory of body length increase (repeated measures ANOVA, [infection] $F_{1,71}$ = 3.72, P>0.05; [infection*time] $F_{8,71}$ = 1.15, P>0.05), but the weight of infected fish (including parasite weight) rose more quickly

Weeks post-exposure / sham exposure

0 2 4 6 8 10 12 14 16

a)

10mm

b)

Fig. 1. Dorsal profiles of a representative control (sham-exposed) and experimentally infected stickleback photographed at 14d intervals over the 16-week study

than the weight of controls (r-m ANOVA, [infection] $F_{1,71}$ = 54.96, P<0.0005; [infection*time] $F_{8,71}$ = 3.17, P=0.006). Conversely, the weight of experimentally infected fish (excluding plerocercoid weight) rose more slowly than the weight of controls (r-m ANOVA, [infection] $F_{1,71}$ = 82.15, P<0.0005; [infection*time] $F_{8,71}$ = 4.59, P<0.0005).

Growth trajectories of individual plerocercoids were reconstructed using plerocercoid weights estimated from morphometric analysis of hosts. Approximately sigmoid growth curves were observed for all parasites, but although the slopes of the plerocercoid growth curves from ~40mg-120mg were similar, there was considerable variation in the time taken for plerocercoids to reach a particular weight (e.g. 6-10 weeks to reach 100mg). Maximum plerocercoid size appears to have been reached by 12 weeks post-infection, with little parasite growth occurring after this time.
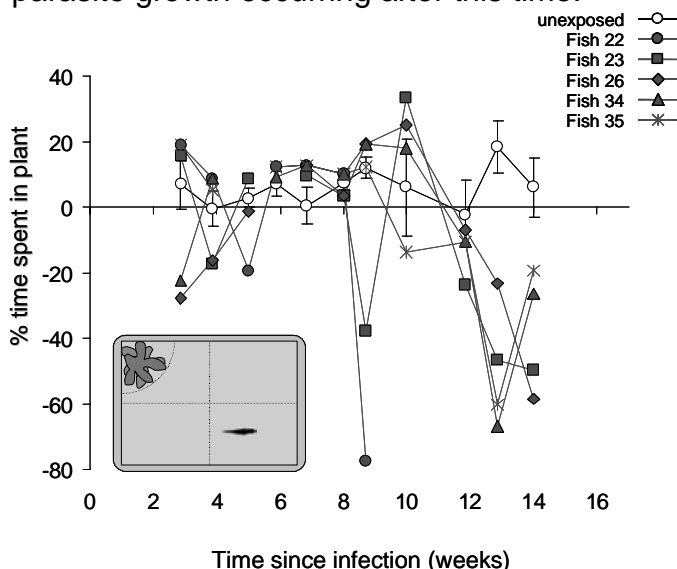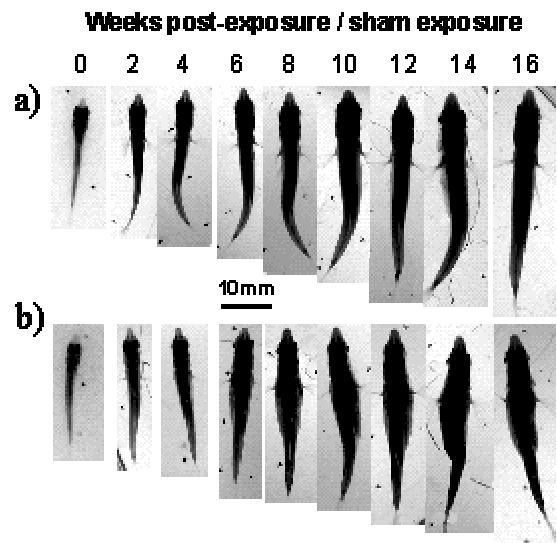

unexposed
Fish 22
Fish 23
Fish 26
Fish 34
Fish 35

% time spent in plant

Time since infection (weeks)

Fig. 2. Shelter use behaviour of control (open symbols; mean ± se) and individual experimentally infected sticklebacks (red symbols) over the 16-week period of the study.

Shelter use behaviour of experimentally infected fish did not differ from that of uninfected individuals over the first 10 weeks post-exposure, but after week 10 infected fish spent considerably less time than uninfected fish in cover (Figure 2). When the data are examined in terms of parasite size rather than time since infection, it is clear that parasites only interfered with normal patterns of behaviour at sizes larger than ~100mg – interestingly, the behaviour of fish 22 was first to be altered by infection, but this was the host that harboured the fastest growing parasite.

The results of escape response studies were complex. Of the 349 simulated

strikes carried out, 219 (63%) resulted in fish undertaking a directional response within the 2s following the release of the model, with fish in the remaining 130 trials (37%) either freezing or not responding to the strike. The maximum velocity attained by sticklebacks in the study during directional responses, over 80ms and 400ms timeframes, are shown in figure 3. As fish grew over the period of the study, the mean $\log_{10}$-transformed maximum escape velocities over both timeframes increased significantly, with $\log_{10}$-transformed mean standard length of the fish in each biweekly screening (Figure 3). Although the slopes of the regression lines describing the relationship between $\log_{10}$-transformed body length and $\log_{10}$-transformed maximum velocity for the two timeframes did not differ significantly ($F_{1,17} = 2.24$, $p = 0.72$), their
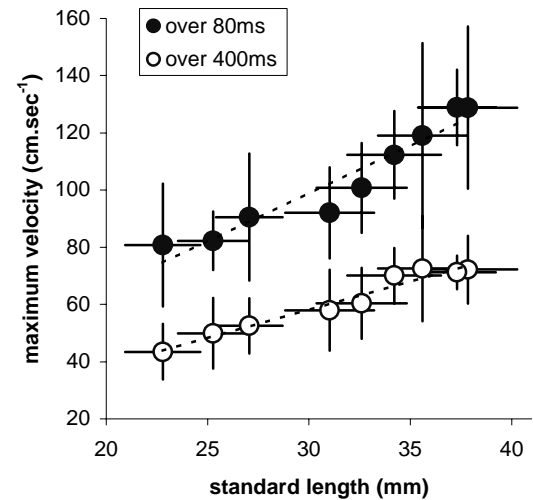

Fig. 3. Maximum escape velocities of sticklebacks measured at 14d intervals, plotted against standard length, over 80ms and 400ms timeframes. Error bars represent standard deviations
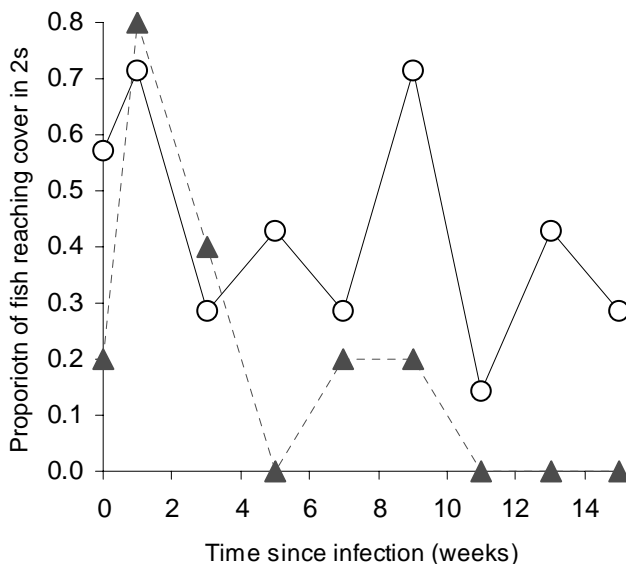
elevations were significantly different, meaning that for a given mean body size, the maximum velocities recorded over 80ms were significantly higher than those recorded over 400ms ($F_{1,17} = 552.77$, $p < 0.0005$). Infection status had no effect on the proportion of fish responding over the total study period (Kruskall-Wallis ANOVA; $H = 3.77$, $df = 2$, $P=0.151$). The proportion of responding control fish reaching cover within 2s did not change consistently over time (Spearman rank correlations; C: $r = -.402$, $p = 0.284$). However, the proportion of responding infected fish that reached cover within 2s declined significantly with time since infection ($r = -0.848$, $p = 0.004$; Figure 4).

Fig. 4. The proportion of responding control (open symbols) and experimentally infected (red symbols) sticklebacks that reached cover within 2s of the simulated avian strike

At autopsy the mass of parasites recovered from infected fish ranged between 0.119g - 0.159g, contributing between 16.6-25.8% of host mass. There was no significant effect of infection on the relative liver weight of female sticklebacks (K-W ANOVA, $H = 0.32$, $P = 0.57$), but infection did have a significant effect on body size corrected perivisceral fat reserves ($H = 5.14$, $P = 0.026$), with infected females having significantly less fat. Unexpectedly, infection was found to have a highly significant effect on sexual development, with infected females having larger ovaries for their body weight than control fish ($H = 8.08$, $P = 0.004$).

Future planned research

This study has been the first to examine behaviour changes in experimentally infected fish. Furthermore, the use of a morphometric technique to track parasite growth non-invasively allowed host behaviour to be examined in terms of parasite development and growth. Our finding that host shelter use behaviour is manipulated when the parasite achieves a weight

of ~100mg suggests that behaviour is not changed as soon as the parasite becomes infective (at ~50mg according to Tierney & Crompton 1992 *J. Parasitol.* **78**, 1049-1054). We are currently investigating how the relationship between plerocercoid size and fecundity might affect optimal strategies of host manipulation and carrying out investigations examining the role of host ration on parasite growth rates.

Acknowledgments

Output from the project

Papers *in press*

Barber, I. & Svensson, P.A. (2003). Effects of experimental *Schistocephalus solidus* infections on growth, morphology and sexual development of female three-spined sticklebacks, *Gasterosteus aculeatus. Parasitology* (in press).

Manuscripts *in preparation*

Barber, I. & Svensson, P.A. Adaptive manipulation of host behaviour by a parasite.

Barber, I., Walker, P. & Svensson, P.A.. Effects of growth and parasitism on behavioural responses to simulated avian predation in three spined sticklebacks

Conference presentations

Barber, I. & Svensson, P.A. Synchrony between parasite development and host behaviour change: An experimental study. Poster presented at the *ASAB Summer Meeting, University of Glasgow, September 2001* and at the *1st European Conference on Behavioural Biology, Muenster, Germany, August 2002.*

Career development

Andreas Svensson (the RA on the project) has since attained a funded PhD studentship position at the Norwegian University of Science and Technology (NTNU) Trondheim.

Expenditure

DV Camcorder (Sony HandiCam TRV320E)	£550
Dedicated PC with DV editing facility	£800
RA hired for 20 weeks @ £160 per week	£3200
Consumables (videotapes, parasite culture media)	£150
Total	**£4700**

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