

BEHAVIOURAL RESPONSES TO SIMULATED AVIAN PREDATION IN FEMALE THREE SPINED STICKLEBACKS: THE EFFECT OF EXPERIMENTAL SCHISTOCEPHALUS SOLIDUS INFECTIONS

by

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Summary

Plerocercoid larvae of *Schistocephalus solidus* are common parasites of three-spined sticklebacks that require the ingestion of stickleback hosts by birds to complete their life cycle. Amongst wild-caught sticklebacks, infection is associated with a reduction in antipredator behaviour; however, to date no study has examined the escape responses of experimentally infected sticklebacks, and thus assigning causality remains difficult. Here, we compare aspects of the antipredator behaviour of five experimentally infected female sticklebacks with sham-exposed controls over a 16 post-exposure week period. During weeks 1-7 post-exposure, the escape responses of infected fish did not differ significantly from those of sham-exposed fish. However, over weeks 9-15, when infected fish had developed plerocercoids of >50 mg — the size at which they become infective to birds — a lower proportion of infected fish performed directional responses and reached cover within 2 s of the strike. Infected fish also performed a lower frequency of ‘staggered dashes’, and a higher frequency of ‘slow swims’, than sham-exposed fish over weeks 9-15. Amongst sham-exposed fish, re-emergence from cover was uncommon throughout the study, but infected fish regularly left cover during weeks 9-15. Our results support those of previous studies examining behavioural change in naturally infected

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fish and, although other explanations remain possible, our finding that behaviour change in experimentally-infected fish is limited to hosts harbouring single infective parasites provides further evidence that the behaviour changes may be parasite adaptations.

Introduction

Schistocephalus solidus is a pseudophyllidean cestode that uses three-spined sticklebacks *Gasterosteus aculeatus* as the second intermediate host in its life cycle. Sticklebacks become infected after ingesting infected copepods (the first intermediate host), and the parasites grow in the fish's body cavity over a period of several months to form large plerocercoids, causing characteristic swelling of the fish's abdomen (Arme & Owen, 1967; Smyth, 1994). The parasite can only attain sexual maturation and reproduce following the ingestion of its stickleback host by a suitable definitive host, typically a bird (Smyth, 1994). Any effects of *S. solidus* on the individual escape responses or other antipredator behaviour of infected sticklebacks therefore have the potential to influence rates of parasite transmission.

Sticklebacks and other small fish that live in shallow waters are under threat from a range of predators including piscivorous fish and birds (Adams *et al.*, 1994; Reimchen, 1994). Although fish inhabiting open water habitats typically form shoals and adopt group responses when threatened by avian predators (*e.g.* Foster *et al.*, 1988; Litvak, 1993; Barber & Huntingford, 1996), those living in shallow vegetated habitats are less reliant on group responses and tend to employ individual antipredator behaviours to escape predators (Hoogland *et al.*, 1957; Huntingford *et al.*, 1994). Avian predators typically induce downward escape responses in fish occupying deeper waters, but because fish inhabiting shallow waters are constrained to performing horizontal escapes. One common strategy employed by individual sticklebacks and other small fish on detecting an overhead stimulus is to flee towards nearby cover and remaining hidden (Huntingford *et al.*, 1994; Godin, 1997; Wootton, 1999). Previous studies have shown that, amongst naturally infected, wild-caught fish, *S. solidus* infected sticklebacks are less likely to respond to model avian predators (Giles, 1983; Ness & Foster, 1999), to flee less far when they do (Godin & Sproul, 1988), and to recover more quickly from the disturbance (Giles, 1983, 1987; Godin & Sproul, 1988; Tierney *et al.*, 1993).

S. solidus requires a period of growth within the stickleback host before it is capable of surviving transmission to the definitive host and producing eggs (at about 50 mg; Tierney & Crompton, 1992). Therefore, if changes in host behaviour result from adaptive manipulation by parasites we would not expect to see them until parasites attain this infective size. Although behaviour changes appear to relate to some extent to the relative weight of the parasite load (Godin & Sproul, 1988; Tierney *et al.*, 1993), the use of naturally infected fish in these studies does not control for previous predator experience or the influences of co-infecting parasites, including other *S. solidus* plerocercoids. Given that experimental infection techniques are well established (Smyth, 1994; Aeschlimann *et al.*, 2000; Arnott *et al.*, 2000; Barber & Svensson, 2003), an alternative, more powerful way to test the effects of *S. solidus* infection status on the antipredator responses of stickleback hosts is to examine changes in behaviour following controlled experimental infection. This has the added advantage that, since fish can be infected with a single plerocercoid, host behaviour changes can be linked unambiguously to patterns of growth and development of individual parasites.

In this paper we report on the escape responses and post-escape shelter use behaviour of lab-bred and reared three-spined sticklebacks following either experimental infection with infective stages of *S. solidus*, or sham-infection. Behaviour was screened at two-week intervals over a 16-week post exposure/sham-exposure period, to track temporal changes in the behaviour of experimentally infected fish compared to control individuals. As the 16-week period spans the growth phase of the parasite (Barber & Svensson, 2003), this approach allows us to link changes in host antipredator behaviour to parasite infectivity to the next hosts on the lifecycle, and therefore examine the likely (parasite) fitness consequences of host behavioural manipulation.

Materials and methods

Experimental fish

Adult three spined sticklebacks *Gasterosteus aculeatus*, caught from an upland reservoir in mid-Wales, UK (Llyn Frongoch, 52°21'N, 3°52'W; 280 m altitude), were brought into breeding condition in laboratory aquaria by manipulating light and temperature regimes. Stickleback fry collected from numerous natural spawnings in laboratory aquaria were maintained in 100-l stock tanks, and after a period of early growth in the lab, 40 size-matched fish (mean \pm SD wet weight, 0.13 \pm 0.03 g) were selected from stock tanks for the study.

Experimental infections

Copepods were exposed to a single infective coracidium following an established protocol (Arnott *et al.*, 2000; Barber & Svensson, 2003) and screened after 6 and 27 d to ensure that infections had been successful. Thirty of the selected fish were fed a single experimentally infected copepod, ensuring that all exposed fish could develop a maximum of one plerocercoid. The remaining 10 fish (sham-exposed controls) were fed a non-parasitised copepod. There was no significant difference in the size (weight) of fish that were fed infected and control copepods ($t = 0.47$, $p = 0.65$). Following exposure/sham-exposure the 40 fish were housed separately in individual 12-l tanks within a filtered recirculating system (Ali & Wootton, 2001), and maintained at $18 \pm 0.5^\circ\text{C}$ on a 11L:13D photoperiod. Fish were fed live cultured whiteworms (*Enchytraeus* sp.) throughout the study at a ration of 8% body weight per day.

Escape response trials

The escape responses of all 40 fish in the experiment were recorded on the day preceding parasite exposure/sham-exposure (week 0) and after 1, 3, 5, 7, 9, 11, 13, and 15 weeks post-treatment. Individual fish were removed from their home tanks in the recirculating system and transferred to a settling chamber in the centre of the experimental arena (0.5×0.5 m, water depth: 8 cm; Fig. 1a). After allowing the fish to settle for five minutes, or 60 s after the fish resumed normal swimming activity (whichever was sooner), the settling chamber was raised slowly, to avoid startling the fish. Once fully raised, the 'striking heron' model was released, and allowed to 'strike' just above the water surface immediately above the fish (Fig. 1b), before being immediately retracted. The immediate responses of fish to the stimulus were recorded at 25 frames s^{-1} using a digital video camera (Sony TRV-320E) positioned above the tank, for subsequent analysis of escape responses (see below). Movements of the fish into or out of plastic plants, positioned at the corners of the experimental arena, and around the arena were recorded manually for 5-min following the simulated attack. At the end of the trial, the fish was removed and returned to its home tank within the recirculating system.

Video analysis of escape responses

Digital videotapes were replayed on a video monitor via a PC equipped with hardware and software for digital video editing (Studio DVplus™, Pinnacle Systems Inc., Braun-schweig, Germany), which facilitated frame-by-frame analysis. The movements of individual fish over the 2 s (50 frame) period following each simulated strike were tracked and analysed using the ImageTool™ image analysis software package (developed by the University of Texas Health and Science Center at San Antonio, and available on the internet at <http://ddsdx.uthscsa.edu/dig/itdesc.html>). We noted whether each fish made a directional response in the 2 s following the strike, or whether there was no response or a 'freeze' response. Directional responses were assigned to one of four discrete types ('dash', 'staggered dash', 'jump' or 'slow swim'; see Fig. 2 for graphical representations) using unambiguous criteria (Table 1).

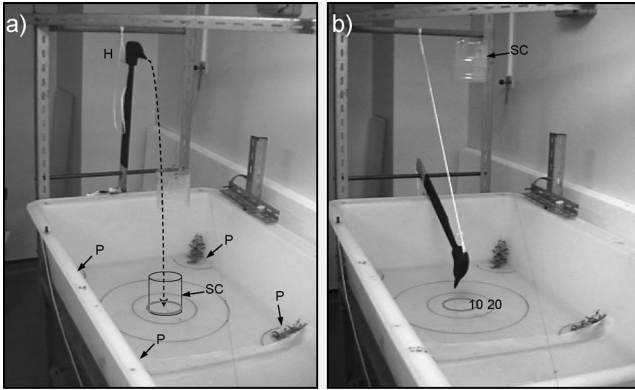


Fig. 1. The experimental arena tank used in the escape response trials. Individual sticklebacks were introduced to the settling chamber (SC), which was then lifted by a remote mechanism. The heron model (H) was released and allowed to fall so that the beak just touched the water surface (Fig. 1b), and the escape responses of the fish were videotaped. A plastic plant (P) was positioned in the corner of the experimental arena, which measured 0.5×0.5 m. '10' and '20' denote 10 cm and 20 cm diameter circles around the strike zone.

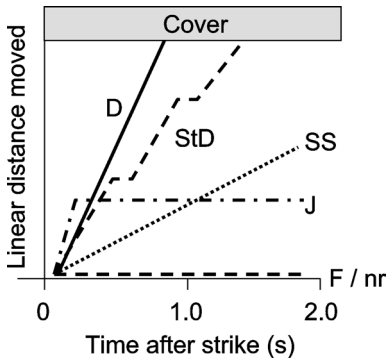


Fig. 2. Graphical representation of the five response types observed following the simulated avian strike. D = dash; StD = staggered dash; SS = slow swim; J = jump; F/nr = Freeze/no response (see Table 1 for precise definitions).

Statistical analysis

Relationships between time since exposure/sham-exposure and the proportional frequency of fish in each group performing various types of escape response were examined using Spearman's rank correlation (Siegel & Castellan, 1988). As *Schistocephalus solidus* takes approximately 8 weeks to reach a size of >50 mg in infected fish fed 8% body weight per day (Barber & Svensson, 2003), changes in host behaviour that are parasite adaptations are more likely to occur during weeks 9-15 than earlier in the infection period. For analysis purposes, trials were therefore split into early post exposure/sham-exposure (weeks 1-7) and

TABLE 1. *Criteria defining the various types of escape response observed amongst sticklebacks following release of the 'striking heron' model*

Response type	Description
'Freeze'/no response	Fish does not move from pre-strike position for at least 2 s following strike
'Jump'	Fish darts from the point of impact but stops before reaching cover and 'freezes' until end of 2 s analysis period
'Dash'	Fish swims directly to cover within 2 s; movement between every consecutive frame of video until cover is reached
'Staggered dash'	Fish swims to cover within 2 s in a series of bursts; no movement between at least two consecutive frames of video
'Slow swim'	Fish swims slowly away from point of impact and does not reach cover within 2 s

late post exposure/sham-exposure (weeks 9-15). Differences in the proportion of infected and sham-exposed fish performing each type of escape response during early and late periods were then tested using Friedman two-way analysis of variance by ranks (Siegel & Castellan, 1988), using 'week' as the blocking variable. To examine the effect of response type on the time taken to reach cover, data from sham-exposed fish only were used, and a Kruskal-Wallis nonparametric ANOVA was performed, followed by *post-hoc* pairwise comparisons (following Siegel & Castellan, 1988). Fish not reaching cover within 300 s were assigned this value as a conservative estimate.

Results

Experimental infections

Infections developed in 5 of the 30 fish fed infective *Schistocephalus solidus* procercoids. As expected, at autopsy all infected fish harboured single pleurocercoids (mass range: 0.110-0.159 g). All infected fish were females, reflecting a strong female bias amongst all fish in the study. Because of the low numbers of males and the possibility of gender differences in antipredator behaviour, we concentrate on the escape behaviour of females in the three exposure groups; sham-exposed control females ($N = 7$), exposed-uninfected females ($N = 20$ females) and infected females ($N = 5$).

Escape responses: general patterns

Fish performed directional responses within 2 s of the release of the model heron in 182 (64%) of the 286 simulated avian strikes analysed. In the remaining 104 trials (36%) fish either performed a non-directional response,

i.e. they either 'froze' in response to the strike, or did not respond to the strike.

Temporal changes in escape responses

There was no relationship between time since exposure/sham-exposure and the proportion of fish in any exposure group making directional responses to the heron model (Spearman's rank correlations; sham-exposed: $r_s = -0.044$, $p = 0.23$; exposed-uninfected: $r_s = -0.75$, $p = 0.20$; infected: $r_s = -0.03$, $p = 0.95$; Fig. 3a). However, over weeks 9-15, a slightly, but significantly, lower proportion of parasitised fish performed directional responses to the strike compared to sham-exposed fish (Friedman ANOVA $S = 4.00$, $df = 1$, $p = 0.046$; Fig. 3c). There was no relationship between time since exposure/sham-exposure and the proportion of sham-exposed or exposed-uninfected fish reaching cover within 2 s (Spearman's rank correlations; sham-exposed: $r_s = -0.402$, $p = 0.284$; exposed-uninfected: $r_s = -0.509$, $p = 0.162$; Fig. 3b). However, the proportion of infected fish that reached cover within 2 s decreased significantly over time (Spearman's rank correlation; $r_s = -0.848$, $p = 0.004$) and a significantly lower proportion of infected fish reached cover within 2 s over weeks 9-15 than sham-exposed fish (Friedman 2-way ANOVA $S = 4.00$, $df = 1$, $p = 0.046$; Fig. 3d).

When directional responses were separated by response type, further patterns emerged. Amongst sham-exposed and exposed-uninfected sticklebacks, staggered dashes were the most commonly observed directional responses, followed by jumps, dashes and slow swims (the least common response) and the proportional frequency of all response types was unrelated to time since exposure/sham-exposure (Spearman rank correlations; all $p > 0.05$; Fig. 4). However, the proportion of experimentally infected fish performing slow swims increased significantly over the post-infection period ($r_s = 0.806$, $p = 0.009$; Fig. 4d). Over weeks 1-7 there was no difference in the proportional frequency of any of the four directional response types performed by experimentally infected fish compared to sham-exposed fish (Friedman 2-way ANOVA, all $df = 1$, S range = 0.00-2.00, p range = 0.16-1.00; Fig. 5). However, over weeks 9-15, experimentally infected fish performed a significantly lower proportion of staggered dashes ($S = 4.00$, $df = 1$, $p = 0.046$; Fig. 5b), and a significantly higher proportion of slow swims ($S = 4.00$, $df = 1$, $p = 0.046$; Fig. 5d), than sham-exposed fish.

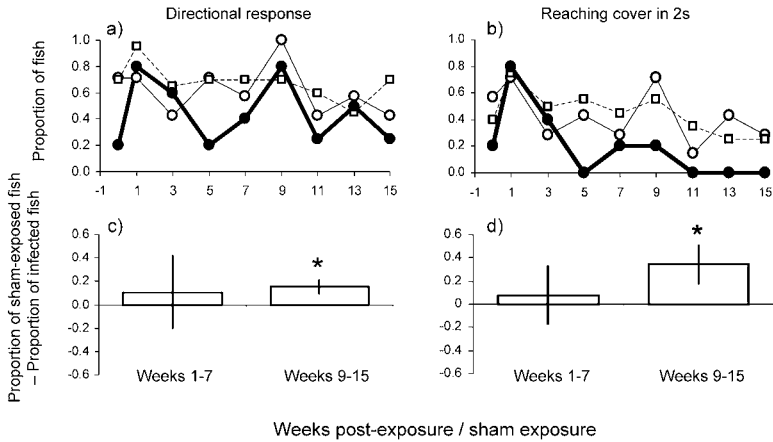


Fig. 3. The proportion of sham-exposed (○), exposed-uninfected (□) and experimentally infected (●) sticklebacks that (a) performed directional escape responses and (b) reached cover within 2 s of the avian strike, over the 16-week study. Histograms show the mean (\pm SD) difference in the proportion of sham-exposed and infected fish (c) performing directional responses and (d) reaching cover within 2 s, grouping responses from weeks 1-7 and 9-15 together. Asterisks denote differences that deviate significantly from zero ($p < 0.05$).

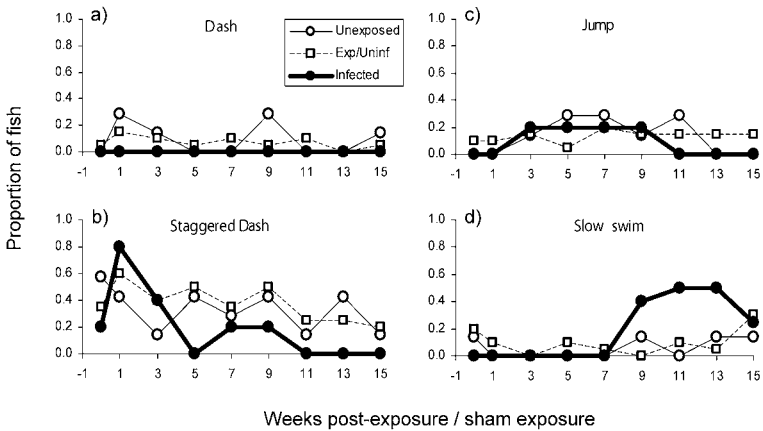


Fig. 4. The proportion of sham-exposed (○), exposed-uninfected (□) and experimentally infected (●) sticklebacks performing (a) dash, (b) staggered dash, (c) jump and (d) slow swim responses to the avian strike over the 16-week period of the study.

Shelter use

Fish entered the cover provided by the plastic plants during the 5-minute post-strike period in 219 (77%) of the 286 trials analysed. Response type had

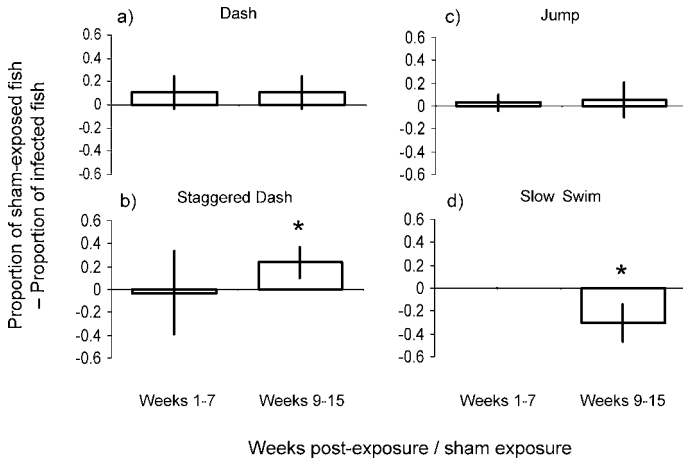


Fig. 5. Histograms showing the mean (\pm SD) difference in the proportion of sham-exposed and infected fish performing (a) dash, (b) staggered dash, (c) jump and (d) slow swim responses to the avian strike, grouping responses from weeks 1-7 and 9-15 together. Asterisks denote differences that deviate significantly from zero ($p < 0.05$).

a significant effect on time taken to reach cover (Kruskal-Wallis ANOVA, $H = 46.8$, $df = 4$, $p < 0.0001$), with fish performing dashes and staggered dashes reaching cover most quickly, followed by those performing slow swims and jumps. Unsurprisingly, those fish initially performing freeze/no responses ultimately took the longest to reach cover (Fig. 6).

The majority (82%) of fish that reached cover remained hidden until the end of the trial, and the proportion of both sham-exposed and exposed-uninfected fish that re-emerged decreased significantly over the 16-week study period (Spearman's rank correlation, sham-exposed, $r_s = -0.803$, $p = 0.009$; exposed-uninfected, $r_s = -0.814$, $p = 0.008$; Fig. 7a). The relationship between the proportion of experimentally infected fish re-emerging from cover and time post-infection was more complex, with a marginally significant decrease over weeks 0-7 ($r_s = -0.866$, $p = 0.058$), and a significant increase between weeks 7-15 ($r_s = 0.975$, $p = 0.005$). Sham-exposed and exposed-uninfected fish that did emerge from cover typically did so only after a prolonged period of hiding, whereas in the later stages of infection (weeks 11, 13 and 15) infected fish commonly left cover after just a few seconds (Fig. 7b).

The only instances of fish leaving cover to re-enter the strike zones were amongst infected fish in the latter stages of the study. In weeks 13 and 15,

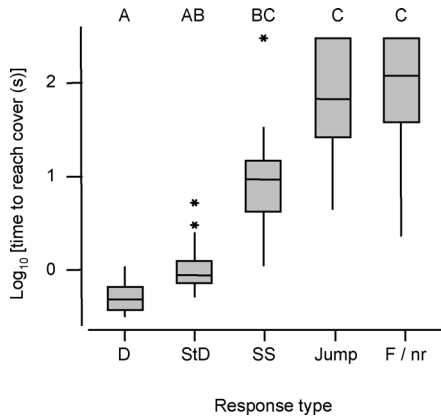


Fig. 6. Boxplots showing the time taken by sham-exposed fish performing each of the five response types to reach cover. Horizontal bars denote medians; boxes show interquartile range; vertical lines show 95% confidence limits; asterisks show outliers. Pairs of response types that do not share letters (A, B or C) differ significantly following *post-hoc* tests ($p < 0.05$).

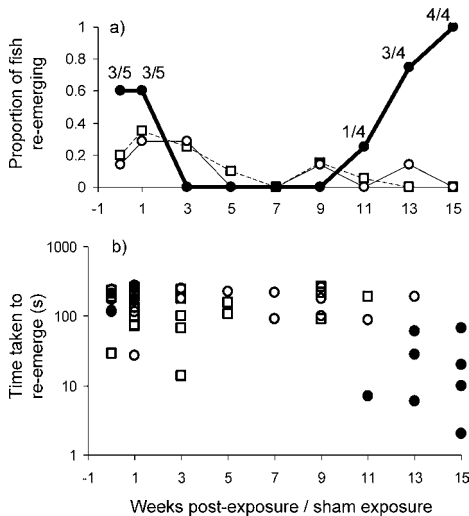


Fig. 7. (a) Line graph showing the proportion of sham-exposed (\square), exposed-uninfected (\circ) and experimentally infected (\bullet) fish reaching cover that subsequently re-emerged within five-minutes of the strike over the 16-week study period. Absolute numbers of infected fish involved are given above data points. (b) For those fish that did re-emerge within five minutes of the strike, the time taken to re-emerge after first entering cover are shown.

respectively, two of three, and three of four, re-emerging parasitised fish entered the 20 cm strike zone, with one and two of these fish entering the 10 cm zone.

Discussion

Previous studies have demonstrated a relationship between *Schistocephalus solidus* infection and impaired antipredator behaviour of host sticklebacks, with infected fish being less likely to respond to, and recovering more quickly from, overhead stimuli (Giles, 1983; Tierney *et al.*, 1993; Ness & Foster, 1999) compared to non-infected fish.

However, because these studies were all carried out on wild caught, naturally infected fish it is difficult to demonstrate causal relationships. In the present study, we repeatedly tested the antipredator behaviour of individual laboratory-bred fish over a 16-week period following experimental infection with single *S. solidus* parasites, and compared their behaviour to sham-exposed controls. Despite small sample sizes, which resulted from the low success rate of experimental infections, our results provide further evidence that the effects of *S. solidus* on the escape responses of sticklebacks are delayed until parasites become infective to the next host. Although there was no detectable effect of infection on behaviour over weeks 1-7, during weeks 9-15 — when, under the imposed host ration, plerocercoids had reached an infective size of >50 mg (Barber & Svensson, 2003) — infected fish were less likely to make directional responses to the overhead stimulus, less likely to reach cover within 2 s, less likely to perform staggered dash responses (the most common escape response amongst non-infected fish), and more likely to undertake slow swims than sham-exposed fish.

Other studies have previously identified a link between the magnitude of *S. solidus* load and host behaviour change. Giles (1983) and Godin & Sproul (1988) both showed that, amongst naturally infected fish, those harbouring relatively heavier parasite loads (expressed as % body weight) resumed feeding more quickly following simulated avian attacks. Godin & Sproul (1988) additionally showed that more heavily infected fish fled less far. However, both studies used a high proportion of fish harbouring multiple infections (mean [range] number of plerocercoids per fish: Giles, 1.76 [1-7]; Godin & Sproul, 2.3 [1-7]) and the use of multiply infected fish creates problems for

interpretation. The essence of the problem is that in these studies a heavy infection (expressed as % body weight) may be generated by either a single, large, infective plerocercoid or by several co-infecting plerocercoids, which may or may not include an infective worm. Thus demonstrating a link between host behaviour change and total plerocercoid weight in multiply infected fish — without information on the size distribution of individual plerocercoids — does not shed light on the adaptive nature of any behavioural change.

Tierney *et al.* (1993) examined the post-strike antipredator behaviour of naturally infected fish harbouring plerocercoids <50 mg and >50 mg, and found behaviour to be changed only in fish harbouring infective worms. Although it is unclear whether any of these fish harboured multiple infections, their study does support the hypothesis that behaviour changes are restricted to the hosts of parasites that would benefit from predation by birds. By tracking temporal changes in the behaviour of individual hosts, experimentally infected with a single plerocercoid, our results add to these findings, and provide further evidence that the onset of host behaviour change may be linked to parasite infectivity. However, our results are subject to two limitations. Firstly, our low sample size preclude more rigorous statistical analyses that could otherwise examine more accurately examine subtle temporal changes in behaviour. Secondly, all infected fish were female and it is possible, though we think unlikely, that infections might affect the escape responses of hosts in a sex-specific manner. Further studies involving larger numbers of experimentally infected fish are required to resolve these issues.

Previous studies have not distinguished between the various escape response types of *S. solidus* infected sticklebacks, recording all directional movements as 'jumps' (Giles, 1983) or 'responses' (Ness & Foster, 1999). By separating directional responses into discrete types, we were able to examine in more detail some of the changes associated with infection. 'Staggered dashes' were the most commonly observed directional responses amongst non-infected fish, but were not performed by parasitised fish during the latter stages of infection. As they incorporate an unpredictable protean element that predators find confusing (Schall & Pianka, 1980; Driver & Humphries, 1988), staggered dashes are likely to be effective responses, but may not be available to heavily-infected fish if infection reduces mechanosensory performance. 'Slow swims' were only commonly observed amongst infected fish during the last three weeks of the study. Such responses

would allow sufficient time for an initially unsuccessful predator to recover, re-locate the fish and mount a secondary attack, and are thus not expected to be effective. Approximately one third of all attacks resulted in 'freezes' or 'non-responses'. 'Freezing' may be an effective response of cryptically coloured prey occupying visual complex habitats, particularly when predators are close and the chances of performing a successful escape are low (Godin, 1997). On the other hand, failure to respond to an attacking predator may be a fatal and final 'mistake'. Unfortunately, we were unable to distinguish 'freeze' responses (characterised by erection of spines, cessation of ventilation movements etc.; Metcalfe *et al.*, 1987; Huntingford *et al.*, 1994) from non-responses in our video recordings. Further studies examining the efficacy of the different responses against a variety of susceptible and non susceptible host predators are required to determine the consequences of altered host for parasite transmission and hence the likelihood of adaptive parasite 'manipulation' (see also Mouritsen & Poulin, 2003).

Non-infected fish in the study significantly reduced the frequency with which they left cover over the course of the study, though whether this was due to ontogenetic factors or learning is unclear. Conversely, infected fish dramatically increased their frequency of re-emergence during the latter periods of the study. This, and their reduced latency to re-emergence, supports previous findings documenting the speed of recovery of parasitised fish following avian attack (Giles, 1983; Godin & Sproul, 1988; Tierney *et al.*, 1993). As studies on food-deprived, non-parasitised fish typically find similar effects (*e.g.* Gotceitas & Godin, 1993), the most likely explanation for the reduced shelter use by infected fish is that the nutritional drain imposed by the developing plerocercoid forces the fish to indulge in risky foraging (see also Milinski (1990) and Barber *et al.* (1995)). However, there is evidence from the present study that not all aspects of the altered behavioural responses of *S. solidus* infected sticklebacks may be explained solely in terms of the energetic demand of the parasite. The physiological and morphological consequences of *S. solidus* infection are superficially similar to those incurred by becoming gravid, with growing parasites and developing gonads making energetic demands on fish and swelling the abdomen. It is therefore interesting to compare our results with those of Rodewald & Foster (1998) who examined the escape responses of gravid and non-gravid females. Despite the apparent energetic and morphological similarities between infected and gravid fish, there were clear differences in the types of escape responses

recorded. Whereas heavily infected fish were unlikely to perform 'protean' staggered dashes in our study, Rodewald & Foster (1998) found that gravid females were more likely than non-gravid fish to incorporate protean elements into their escape swims. This comparison strongly suggests that the altered escape behaviour of parasitised fish cannot be explained simply in terms of the energetic burden or body distension.

Manipulation or side effect of infection?

There has been considerable recent interest in examining whether infection-associated changes in host behaviour lead to increased parasite transmission (and are therefore likely to be parasite adaptations) or whether host behaviour changes have neutral/negative consequences for transmission, for example, by increasing susceptibility to non-host predators (e.g. Lafferty & Morris, 1996; Levri, 1999; Webster *et al.*, 2000; Mouritsen, 2002; Edelaar *et al.*, 2003; Mouritsen & Poulin, 2003). In the latter case, host behaviour change may be more likely to be an inevitable side effect of infection than a parasite adaptation. Examining the precise relationship between parasite infectivity and host behaviour change, and identifying the mechanisms by which parasites suppress host antipredator behaviour, is another way of gathering information on the likelihood of adaptive manipulation. Our next challenges are to determine whether temporal aspects of behaviour change in experimentally infected sticklebacks correlate more closely with parasite mass or developmental processes related to infectivity, and to determine the mechanisms by which the parasite suppresses host antipredator behaviour. Investigating the behaviour of sticklebacks experimentally infected with single or multiple parasites may provide routes to tackling to these tantalising questions.

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