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Parasitism and behavioural syndromes in the fish *Gobiomorphus cotidianus*

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Abstract

It is becoming clear that individual animals exhibit behaviours that are repeatable, consistent and predictable across various contexts and time, and that may be correlated to form behavioural syndromes. The influence of parasitism on host behaviour, although well-documented, is only now being examined in a multi-trait context, and behavioural syndromes provide a framework for such investigations. We test the hypothesis that parasites modify host behavioural syndromes by quantifying the relationship between infection by four trematode species and the correlations, repeatability and consistency of three host behaviours (activity, aggression and boldness) in a freshwater fish, *Gobiomorphus cotidianus*, in the presence and absence of a predator odour. There was a significant correlation between activity and aggression, and both these behaviour showed higher values in the presence of a chemical predator cue. Of the behaviours tested, only aggression responses were significantly related to infection with one of the parasites, *Apatemon* sp. Activity, aggression and boldness scores were repeatable across test sessions, but much less so in the presence of a predator cue. Interestingly, individual-level consistency in activity and aggression was significantly associated with infection by *Telogaster opisthorchis*, *Deretrema philippae* and/or *Apatemon* sp., with the direction of the association depending on the behaviour tested. These findings suggest that behavioural syndromes may not only be targets for adaptive host manipulation by parasites, but also that, even if through mere pathology, parasites could act as external agents generating variation in behavioural syndromes.

Keywords

animal personality, behavioural consistency, host manipulation, predation risk, trematodes, trophic transmission.

1. Introduction

Individual animals exhibit behavioural traits that are consistent, repeatable and predictable across various contexts and time, and that may be correlated

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to form behavioural syndromes (Sih et al., 2004; Bell, 2007; Réale et al., 2007). Consistent differences in individual traits such as boldness, aggressiveness or sociability generate distinct animal personalities, and may play a role in ecological processes, influencing niche expansion, dispersal and social organization (Bell, 2007; Réale et al., 2007; Kortet et al., 2010). Distinct traits that are consistent across situations may result in tradeoffs for individuals. For example, a bolder individual could benefit in certain situations, such as when boldness allows increased foraging time, but not in other situations, such as when the organism's risk of predation is increased (Smith & Blumstein, 2008). However, the extent to which external factors may influence the consistency of these personality traits across various contexts has not yet been elucidated.

Parasitism may be one such factor. Infection by parasites changes the 'state' of an individual and, thus, possibly the expression of its personality (Dall et al., 2004). Parasites can alter host behaviour either as a pathological side-effect of infection, or in ways that benefit their transmission to other hosts (Poulin, 1995; Moore, 2002). In particular, parasites requiring trophic transmission from a prey intermediate host to a predatory definitive host often manipulate their intermediate host's behaviour to increase its susceptibility to predation (Lafferty, 1999; Thomas et al., 2005; Poulin, 2010). Host manipulation can be far more complex than a single change in colour or behaviour, often involving alteration of several traits at the same time (Thomas et al., 2010). By affecting an intermediate host along several phenotypic dimensions, a trophically-transmitted parasite may significantly increase the likelihood of predation by its definitive host (Thomas et al., 2010). Whether through mere pathology or via an adaptive manipulation, parasites, therefore, have the potential to modify behavioural syndromes in many ways (Barber & Dingemanse, 2010; Kortet et al., 2010; Poulin, 2010).

For instance, the correlations among behavioural traits that constitute behavioural syndromes within populations may be altered by parasites. Earlier research on the effects of infection on behaviour has mainly focused on single host traits, and the focus is only now shifting toward understanding how parasitic manipulation affects suites of behaviour traits and correlations among them (Barber & Dingemanse, 2010; Coats et al., 2010). Parasites could either weaken or strengthen correlations between traits, or cause shifts in behavioural syndromes (Poulin, 2010). Parasitism may even result in new correlations between traits, or allow for the persistence of certain trait correlations within populations (Barber & Dingemanse, 2010; Kortet et al., 2010). Selection may indeed favour parasites capable of decoupling particular traits that act to protect against predators, if this results in significant increases in trophic transmission (Kortet et al., 2010; Poulin, 2010). Parasites may, therefore, play a significant role in shaping the expression of individual personalities, as well as population-wide behavioural syndromes (Barber & Dingemanse, 2010; Kortet et al., 2010; Avilés & Parejo, 2011).

Parasitic infection may affect not only correlations between behaviours, but also the repeatability of behaviours, i.e., the consistency of behavioural traits shown by individual hosts. It is possible to measure the level of repeatability of behaviours within populations, as well as the consistency of an individual's behaviour in various contexts (Réale et al., 2007; Bell et al., 2009). Few studies to date, however, have actually examined how the repeatability and correlations of behaviours are altered by parasitic manipulation. One might expect that the physiological stress imposed by parasites might result in greater variability (lower repeatability) in behavioural responses. In an amphipod used as an intermediate host by trematodes, infection strengthened correlations between behaviours but reduced the repeatability of behaviours in infected hosts (Coats et al., 2010). This may have serious fitness consequences for the host, though it may benefit the parasite if it increases its likelihood of transmission to its predatory definitive host by interfering with anti-predator defences.

In the context of trophic transmission, modifications of host behaviour may only be beneficial for the parasite in the presence of predatory definitive hosts. Therefore, parasite infection and predation risk may have interactive effects on behavioural syndromes. In the presence of predation cues, many animals may alter their behaviours in order to reduce predation risk. Predation cues have been shown to strengthen the correlations between certain traits in fish, suggesting that particular correlations may be adaptive in some situations. For example, in the three-spined stickleback (*Gasterosteus aculeatus*) a correlation between boldness and aggression was present only during exposure to predation (Bell & Sih, 2007). Predation cues may not only influence correlations between traits, but may also result in whole behavioural syndromes becoming apparent only in the presence of predators. A syndrome involving aggressiveness, activity and boldness in sticklebacks was revealed only in the presence of predators (Dingemanse et al., 2007).

If parasitism affects the behavioural correlations found in other contexts, it may very well have impacts in predation situations. This should have consequences for the likelihood of an individual to survive predator encounters, thus highlighting the potential fitness consequences of parasitism through behavioural syndromes.

Here, we investigate the relationship between parasitic infection and a behavioural syndrome in a freshwater fish, the common bully *Gobiomorphus cotidianus* (Eleotridae), focusing on three behavioural traits: activity, aggression and boldness. The repeatability of, and correlations among, these traits were examined in relation to infection levels by four parasite species, in the presence and absence of a predator odour. We tested the general hypothesis that parasites are significantly associated with behavioural syndromes. More specifically, we tested the predictions that: (i) fish with heavy parasitic infection have significantly altered behaviours and personality; (ii) a predation cue influences fish behaviour, acting as a signal for the presence of a definitive host and interacting with parasite infection to determine anti-predator behaviour and (iii) behavioural consistency (i.e., individual variation for a particular behaviour) is weaker in heavily-infected fish.

2. Materials and methods

2.1. *Study species and collection site*

Common bullies, *G. cotidianus*, are small benthic fish widespread in New Zealand lakes (McDowall, 1990). They are very abundant, and are a major prey item for introduced salmonid fishes as well as New Zealand longfin and short-finned eels (*Anguilla dieffenbachii* and *Anguilla australis*). Bullies are found mainly in the littoral zone of lakes, feeding on benthic invertebrates; they are loosely solitary, with males becoming highly territorial during the breeding season. They harbour several species of helminth parasites, and the four most commonly found parasites were considered in this study. They are the trematodes *Apatemon* sp., *Telogaster opisthorchis*, *Stegodexamene anguillae* and *Deretrema philippae*.

Young bullies between 25 and 55 mm in length were caught using seine nets on the shore of Lake Waihola (46°00′S, 170°06′E), in Otago, New Zealand, during March 2011. They were immediately transported back to the lab in aerated transport tanks for processing. All procedures for fish capture, handling, recording and euthanasia were approved by the Otago University Animal Ethics Committee (protocol No. 09/11).

2.2. Parasite lifecycles

Apatemon sp., *Telogaster opisthorchis* and *Stegodexamene anguillae* have complex lifecycles involving two intermediate hosts, a gastropod as the first intermediate host and the common bully (*G. cotidianus*) as the second intermediate host. These three parasite species all infect bullies via skin penetration, after infective larvae (cercariae) released from the gastropod host make contact with the fish. They then encyst as metacercariae within the fish's body cavity, muscles, gonads, or other tissues. *Apatemon* sp. uses a bird as definitive host and, therefore, requires avian predation on bullies to complete its lifecycle. Adult worms have been confirmed by DNA sequencing to occur in mallard ducks (*Anas platyrhynchos*) and New Zealand scaup (*Aythya novaeseelandiae*) (B. Presswell, pers. commun.). *Telogaster opisthorchis* and *S. anguillae* both utilise predatory eels (*Anguilla* sp.) as their definitive host (Macfarlane, 1945, 1951). Typically, all bullies in natural populations harbour at least a few metacercariae of these three trematode species, with numbers of parasites per fish sometimes exceeding 100 or even 200 (Herrmann & Poulin, 2011). *Telogaster opisthorchis* is known to reduce the responses of upland bully (*Gobiomorphus breviceps*) to the sight of a predator and, thus, it is likely that the behaviour of common bullies may also be affected (Poulin, 1993). This trematode species also causes severe malformations and high mortality among young galaxiid fishes (*Galaxias anomalus*), suggesting it acts as a strong selective agent on young fish (Kelly et al., 2010). Even in larger fish, infection from encysting metacercariae such as those of *Apatemon* sp., *S. anguillae* and *T. opisthorchis*, may result in significant physiological stress, potentially leading to death and abnormal behaviour, as seen in other systems (Ferguson et al., 2011).

In contrast, adult *Deretrema philippae* are found in the gallbladder of common bullies, and use bullies as their definitive host; fish acquire the parasite when they ingest crustacean intermediate host harbouring the parasite's encysted larval stages, i.e., metacercariae (Holton, 1983). Adult *D. philippae* mate within the host's gall bladder, and pass out their eggs in fish faeces. Therefore, unlike the other three trematode species, *D. philippae*'s transmission does not necessitate predation on bullies, and its influence on bully behaviour is expected to be minimal and/or only associated with pathology.

2.3. Pre-experimental maintenance and tagging

Fish were measured to the nearest millimetre, and divided into 3 size groups (26–35, 36–45 and 46–55 mm), each with 24 fish (total $N = 72$). Within each size group, fish were divided into groups of eight and maintained throughout the experiment in 60-l opaque tanks, each with an individual filter and bubbler. All fish were treated for fungal skin infection using Profurin (1 g/100 l) for 30 min and given a 5-min saltwater bath to eliminate any monogenean ectoparasites, as lethal infections may occur in untreated captive bullies.

Water was gradually (over a few days) changed from fresh lake water to a mixture of 67% spring water and 33% saltwater to match the salinity of the natural habitat, and to prevent fungal growth. Tanks were kept at a temperature of 14°C under a natural 12 h light/dark cycle. Every second day, a third of the water was replaced with a fresh mix of freshwater/saltwater, using a siphon to remove excess food and waste. Fish were fed ad libitum with commercial fish pellets (Reliance Stock Foods, Dunedin, New Zealand) every two days, and each tank had five 10-cm-long (4 cm diameter) PVC pipe sections providing ample refuge space for all fish.

After 3 days acclimatisation, fish were anesthetised individually, using 10 mg/l of the fish anaesthetic tricaine methanesulphonate (MS-222). Once a fish showed no movement and was unable to remain upright, it was rinsed to remove excess anaesthetic and a tag was inserted subcutaneously. This consisted of a 2-mm-long elastomer implant (Northwest Marine Technology, Shaw Island, WA, USA); two tag colours and 4 body locations allowed for 8 unique tags, one for each fish in a group. Fish were allowed to recover in new water (67% spring water, 33% saltwater), then returned to their tanks for one week before the experiment started. All fish recovered well and none showed any signs of adverse effects.

2.4. Predator odour cue

To simulate the presence of a predator during the second session of behavioural tests (see below), we used water from an eel (*Anguilla australis*) tank. *Anguilla australis* is a natural predator of bullies, and also the definitive host of both *Telogaster opisthorchis* and *Stegodexamene anguilla*. Water was collected daily in 20 l amounts to ensure that it was fresh, and that each group received the same concentration of predator cue. Water was taken from a 3000-l tank containing 120 previtellogenic eels (stocking density 40 kg/m^3) kept at the same salinity as in the experiment (67% spring water, 33% saltwater), and fed on a diet of commercial fish pellets; these eels are stocked in Otago University's Zoology Department for ongoing and unrelated physiological research. This eel-scented water was under constant filtration and daily water changes during eel maintenance.

2.5. Behavioural tests

Three sessions of testing were conducted, with three weeks of recovery between successive sessions. During each session, all fish were tested twice with 7 days between the first and second test day. During the second session, a predator cue was added to the water. For a test, fish ($N = 8$) were gently removed from their tank one by one with a dipnet; each individual was placed into an 8-l container, visually isolated from other fish and the observer by opaque material, with 3 l of fresh freshwater/saltwater mix at the same temperature as the tank water. During the second session of testing, $1/3$ of the freshwater was replaced with eel water to act as a predator cue. Each container was also given one 15-cm-long opaque PVC pipe section as refuge during all tests. Once each fish was placed into its container, a lid was placed gently on top and the fish were given 30 min to acclimatize before testing began; all observations were made from above through that lid, always by the same person. Three behavioural tests were carried out (aggressiveness, boldness and activity), with 1 h between them and their order was randomised for each group. After the last test, the group was immediately returned to their large tank. The tests (see below) follow methods that have now become standard in studies of behavioural syndromes in fish (Dingemanse et al., 2007; Eriksson et al., 2010).

2.6. Behavioural traits

Aggressiveness was measured as the amount of active ‘attacking’ or lunging that the fish targeted at a ‘conspecific’ within a 3-min period. A 15 × 10 cm mirror was placed lengthwise in the container. A cover on the mirror remained for 5 min before it was removed. Once the cover was removed, the fish was allowed 30 s to adjust to the mirror. Then, the amount of aggression exhibited by the fish was scored as the total time the fish spent lunging at or moving against the mirror, over a 3-min period. Aggression was only considered as active movement; thus, any time the fish was alongside the mirror but not actively moving was not counted. At the end of the 3-min period the mirror was removed. Fish never rammed the mirror or made contact with it in ways that could injure them. The use of a mirror to simulate a conspecific has been successfully used in several fish behavioural studies, including a study of aggression in bullies (Hamilton & Poulin, 1995).

Boldness was measured as the time that the fish took to emerge from the refuge after a simulated predator attack. An attack was simulated using a dark metal rod that lunged at the fish, but without making contact, when the latter was outside the refuge. After a single strike, a timer was started once the fish entered the refuge. A maximum of 5 min was allowed for the fish to exit the refuge, at which point it was scored as taking longer than 5 min. When the fish emerged up to 1 cm from the edge of the refuge, it was counted as an exit. If the fish refused to enter the refuge, it was given a score of 0 s.

Activity was measured as the amount of time spent actively moving outside the refuge within a 3-min period. Fish were given 5 min once the container lid was removed before timing started, to reduce any disturbance from lid removal. At any point when the fish was visibly moving (other than opercular breathing movements), it was considered active.

2.7. Dissections and parasite counts

Once all tests were complete, fish were killed via an overdose of the anesthetic MS-222 (10 mg/l, for 15 min) followed by immediate decapitation. Fish were measured (total length) before dissection took place, and sex was determined during dissection. All tissues were thoroughly inspected for parasitic infection, and numbers of parasites of each species were recorded. Cysts of *Apatemon* sp., *Telogaster opisthorchis* and *Stegodexamene anguillae* were present in connective tissue, gonads, liver and muscle tissue. The gallbladder was examined for *Deretrema philippae*, as this is the only site of infection for this parasite. Other parasite species were also found during dissection, but excluded from the analysis because of very low abundance. The larval cestode *Paradilepis* sp. was present in 6 fish that on average harboured 2 cestodes (range 1–6) in their body cavity. The nematode *Eustrongyloides* sp. occurred in 3 fish, each only containing one cyst in the body cavity. Finally, the trematode *Coitocaecum parvum* was found in the intestines of 2 fish containing three and one adult parasites, respectively.

2.8. Statistical analysis

All statistical analyses were carried out using the statistical program R (version 2.13.1; R Development Core Team, 2011); full statistical results and datasets are available upon request. Generalised linear mixed-effects models (GLMM) with a binomial error structure (the logit-link function; see below) were used to examine the effects of parasitic infection (intensity, i.e., numbers of parasites per fish, for each species), day, session, and fish sex and size (fixed factors) on the three behavioural responses, activity, aggression and boldness. Fish identity was set as the random factor to account for correlated structures within the data arising from measurements on the same individuals. The use of cut-off values for the behavioural responses meant that no transformations could improve the distribution of the data. For activity and aggression, a cut-off score of 0 s meant that no activity or aggression had taken place within the 3-min trial. For boldness, the time it took for a fish to exit the refuge after a simulated predator attack was measured, with a higher score indicating a shyer animal and, thus, a score of 300 s was used as the cut-off threshold. For this reason, responses were treated as binary data for binomial GLMMs. This was done by assigning 1 to scores of >0 s and 0 to scores of 0 s for activity and aggression, and 1 to scores of <300 s and 0 to scores of 300 s for boldness. When the results of the GLMMs were compared to those of models using rank transformed responses (i.e., linear mixed-effects models, LMMs; i.e., equivalent to using non-parametric tests), the results were qualitatively very similar. However, the models with the rank-transformed data violated the assumption of normal residuals, mainly due to the excessive numbers of extreme values (e.g., approximately 60% of aggression responses were 0). Therefore, GLMMs on binary transformed data, which fulfilled all the statistical assumptions, were used instead, although the binary coding may have led to some loss of information.

Spearman's rank correlations between the three behaviours were calculated along with 95% confidence intervals (CIs). Means of behaviours for individuals, computed across all trial days and sessions, were used for these calculations. Repeatability estimates for each behaviour were calculated from the binomial GLMMs (described above) using binary data following Nakagawa & Schielzeth (2010), for the overall dataset (six trials over all three sessions), and within each session (two trial days per session). Repeatability was calculated using the R package rptR (http://r-forge.r-project.org/projects/rptr). Confidence intervals for repeatability estimates were calculated using standard error, as described in Nakagawa & Schielzeth (2010). This results in confidence intervals that exceed 0 and 1, unlike other methods for calculating 95% CIs.

To assess the relationship between infection intensities and individual consistency of behaviours over the six trials, the standard deviation of the six behavioural trials for each behaviour in each individual fish was divided by the population standard deviation of that behaviour. This ratio of individual-to-population standard deviation ($\mathrm{SD_i}/\mathrm{SD_p}$) has been proposed as a measure of the consistency of individual behaviour (Réale & Dingemanse, 2010). Repeatability provides a measure of behavioural consistency at the population level; however, using the standard deviation ratio ($\mathrm{SD_i}/\mathrm{SD_p}$) we can examine consistency at the individual level within the population. Our data on the three behavioural traits were heavily skewed, and consequently there were strong correlations between individual means and individual standard deviation for each of the traits (often referred to as 'the mean-variance relationship'; activity: $r_\mathrm{S} = 0.81$, $p < 0.001$; aggression: $r_\mathrm{S} = 0.91$, $p < 0.001$; boldness: $r_\mathrm{S} = 0.59$, $p < 0.001$). To deal with this problem, we used rank-transformation on these traits and calculated standard deviations for individuals and the population. With the standard deviation ratio, based on ranks, the mean-variance relationships were greatly weakened (activity: $r_\mathrm{S} = 0.29$, $p = 0.002$; aggression: $r_\mathrm{S} = 0.55$, $p < 0.001$; boldness: $r_\mathrm{S} = 0.005$, $p = 0.968$) although some remained statistically significant and substantial. We used the rank-based standard deviation ratios as our measure of individual consistency. Linear models (LMs) were used to analyse the effects of infection intensities on the individual consistency of fish behaviours (approximately normally distributed), while controlling for fish sex and size. Finally, Spearman's rank correlations between behavioural consistencies were estimated along with 95% CIs, for all pairs of behaviour.

3. Results

3.1. Parasite prevalence, and fish sex and size

All 72 fish had some level of *Apatemon* sp. infection (range 12 to 772 cysts per fish) and overall *Apatemon* sp. was the most abundant parasite. For the other species, the percentages of fish infected were 96% for *Stegodexamene anguillae*, 93% for *Telogaster opisthorchis* and 58% for *Deretrema philippae*. For all four parasite species, infection intensities generally increased with fish length (Figure 1). The mean numbers of parasites per fish were 218 for *Apatemon* sp., 12 for *S. anguillae*, 11 for *T. opisthorchis* and 1 for *Deretrema philippae*. Fish sizes ranged from 22 to 56 mm, with an average of 38 mm. Thirty-nine fish were female (54%) while 33 were male (46%). Four fish died during the experiment, all between the periods of session one and two.

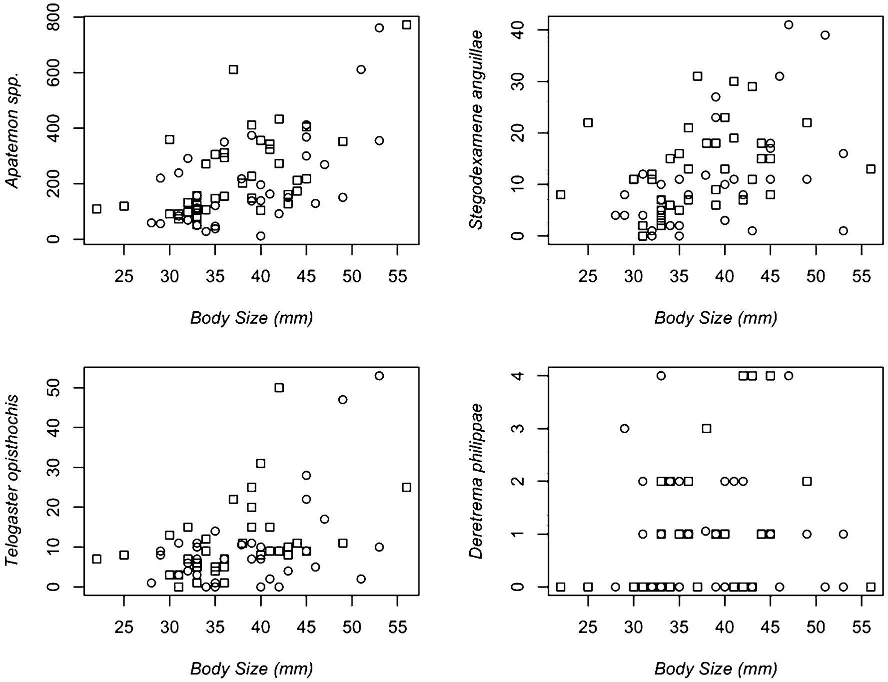

Figure 1. Infection intensity (number of parasites per fish) of the four parasite species, *Apatemon* sp., *Stedodexamene anguillae*, *Telogaster opisthorchis* and *Deretrema phillippae*, in male (circles) and female (squares) common bullies (*Gobiomorphus cotidianus*), as a function of fish body length ($N = 72$).

3.2. Correlations between behavioural traits

Correlations between activity, aggression and boldness are summarised in Figure 2. There was a strong positive correlation between activity and aggression overall ($r_S = 0.49$, $p < 0.001$), whereby individuals that were more active were also more aggressive, and this relationship was also found within each session (note that Cohen's effect size benchmarks for correlation coefficients are $r = 0.1$, 0.3 and 0.5, for 'small', 'medium' and 'large', respectively) (Figure 2). Activity and boldness showed no correlation overall, or in session two and three (all $r_S \leqslant 0.06$ and $p \geqslant 0.523$). In session one however, there was a significant weak to medium negative correlation between activity and boldness, with more active individuals being bolder ($r_S = -0.21$, $p = 0.013$) (Figure 2). Overall and in session one, aggression and boldness had a negative but non-significant correlation ($r_S = -0.22$, $p = 0.062$, and $r_S = -0.15$, $p = 0.069$, respectively). There was no correlation between

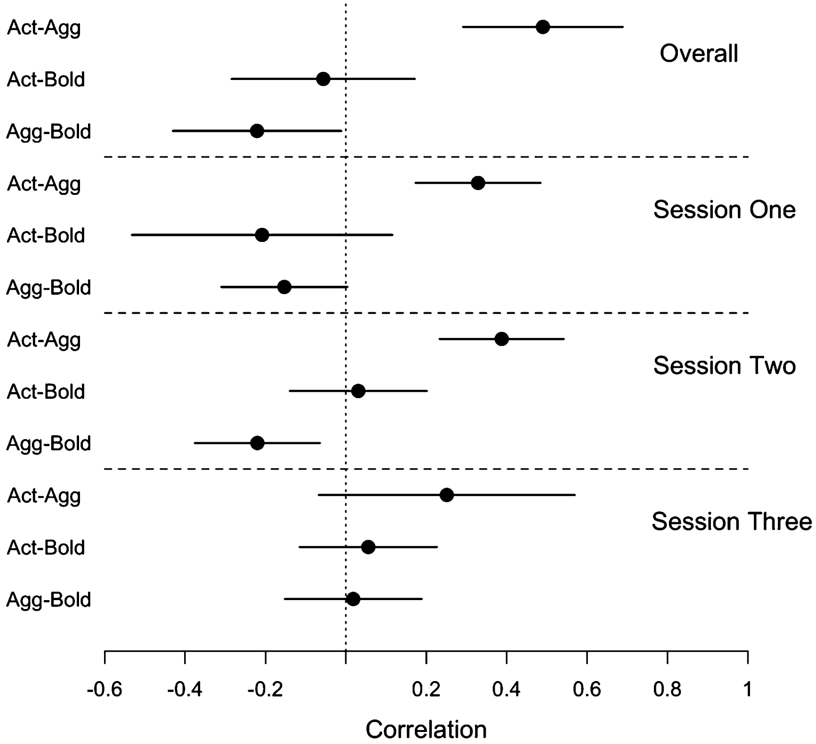

Figure 2. Spearman rank correlation coefficients with confidence intervals among the three host behaviours of common bullies (*Gobiomorphus cotidianus*), overall and within each session. Overall and session one: $N = 72$; sessions two and three: $N = 68$. Act, activity; Agg, aggression; Bold, boldness.

these traits in session three ($r_S = 0.02$, $p = 0.834$). In session two however, when a predator odour was present, this correlation became significant, with more aggressive individuals showing decreased boldness ($r_S = -0.22$, $p = 0.011$) (Figure 2).

3.3. Effects of parasitic infection on behaviour

Generalised linear mixed-effects models (GLMM) were used to analyse the effects of infection intensities, trial day (first or second day in session), session, and fish body size and sex on each of the three behavioural traits (Table 1). There was no statistically significant effect on activity levels from any parasite species or from fish sex; there was however, a significant effect on activity from day, session and fish size. Activity peaked during session

Table 1.
Results of generalised linear mixed-effects models (GLMM) examining behavioural traits of the fish *Gobiomorphus cotidianus* in relation to infection intensity by four parasite species, day (first or second day of testing) and session, and controlling for fish sex and body size ($N_{[\text{individuals}]} = 72$ and $N_{[\text{observations}]} = 410$).

Response	Factor	Estimate	SE	*z*	*p*
Activity	Intercept	1.070	1.023	1.045	0.296
	Day (2nd day)	**0.436**	**0.221**	**1.979**	**0.048**
	Session 2 (with predator odour)	**1.176**	**0.275**	**4.272**	**<0.0001**
	Session 3	**0.753**	**0.274**	**2.748**	**0.006**
	Size	**−0.069**	**0.025**	**−2.730**	**0.006**
Aggression	Intercept	0.628	1.052	0.597	0.551
	Session 2 (with predator odour)	**1.314**	**0.282**	**4.663**	**<0.0001**
	Session 3	**0.899**	**0.282**	**3.183**	**0.002**
	Size	**−0.067**	**0.032**	**−2.108**	**0.035**
	***Apatemon* sp.**	**0.003**	**0.001**	**2.281**	**0.023**
Boldness	Intercept	0.593	0.465	1.275	0.203
	Day (2nd day)	0.527	0.282	1.870	0.062
	Session 2 (with predator odour)	0.532	0.324	1.640	0.101
	Session 3	**0.942**	**0.354**	**2.665**	**0.008**

Only results from the AIC-best model are shown here. Significant predictors are in boldface The sex effect here is the male, since the female effect is included in the intercept, along with day one and Session 1. Note Session 2 and Session 3 above represent the contrasts between Sessions 1 and 2, and between Sessions 1 and 3, respectively.

two, in which a predator odour was present, this large effect being highly significant. Increasing fish size also resulted in decreased activity levels.

Intensity of only one parasite species (*Apatemon* sp.) was found to be significantly related to aggression, with higher intensities being correlated with greater aggression (Table 1). There was also a significant effect from session two, session three and fish size, though no effect was seen from day or fish sex. Session two, when the predator odour was present, showed the largest increase in aggression levels, as was found for activity, followed by session three. Increasing fish size caused a decrease in aggression levels, similar to what was seen in activity.

Observation session was the only factor to affect boldness, with a significant increase in boldness seen during session three; there was no effect from any parasite species (Table 1). Day was close to having a significant effect, however.

3.4. Repeatability of behaviours

The overall repeatabilities for activity, aggression and boldness were all found to be low to moderate (Figure 3). Repeatability within each session was also examined. For session one, activity and boldness had high repeatability ($R = 0.77$ and $R = 0.41$), while that for aggression was low ($R = 0.07$). In session two (when predator odour was present), all three behaviours had very low repeatability (all $R \leqslant 0.14$). For session three, repeatability of activity was low ($R = 0.21$); while for both aggression and boldness it was high ($R = 0.53$ and $R = 0.77$).

3.5. Effects of parasites on the consistency of behaviours

Unlike pairwise correlations among the three behavioural traits (Figure 2), there was little evidence for correlations between pairs of consistency mea-

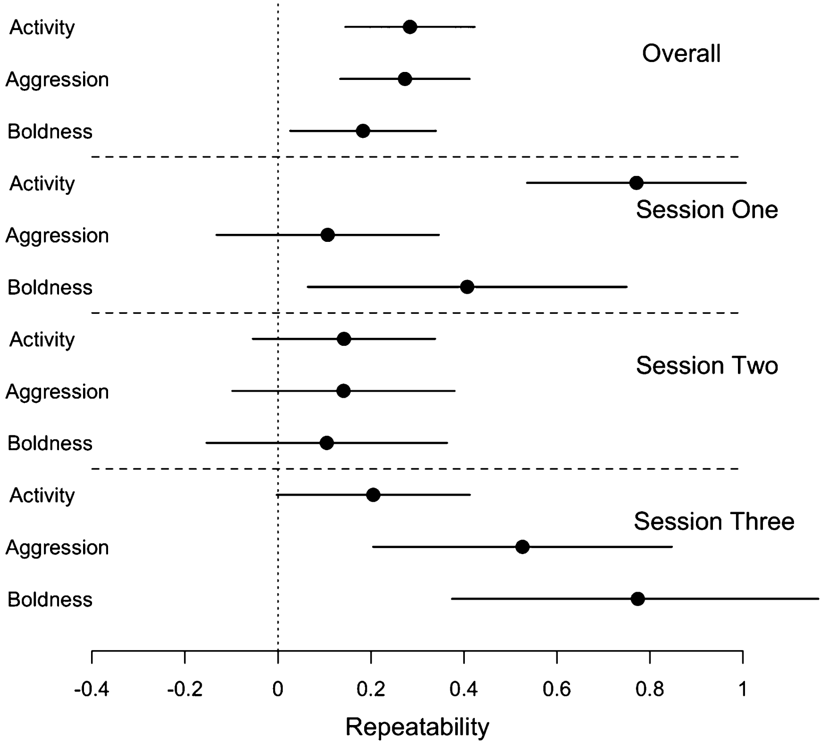

Figure 3. Repeatability estimates and their 95% confidence intervals for the three behaviours (activity, aggression and boldness) of common bullies (*Gobiomorphus cotidianus*), measured overall and within each of the three sessions. Overall and session one: $N = 72$; sessions two and three: $N = 68$.

Table 2.
Results of linear models (LMs) examining the consistency of host behavioural traits in the fish *Gobiomorphus cotidianus*, across all six measurements in relation to infection intensity by four parasite species, and controlling for fish sex and body size.

Response	Factor	Estimate	SE	*t*	*p*
Activity	Intercept	0.298	0.262	1.134	0.261
df = 65	**Body size**	**0.019**	**0.008**	**2.395**	**0.020**
	***Apatemon* sp.**	**−0.0007**	**0.0003**	**−2.180**	**0.032**
	Stegodexamene anguillae	−0.007	0.005	−1.457	0.150
	Telogaster opisthorchis	−0.065	0.035	−1.836	0.071
Aggression	Intercept	0.671	0.061	11.00	<0.0001
df = 68	***Telogaster opisthorchis***	**0.009**	**0.004**	**0.004**	**0.027**
Boldness	Intercept	0.176	0.238	0.738	0.463
df = 68	**Body size**	**0.017**	**0.006**	**2.836**	**0.006**

Only results from the AIC-best model are shown here. Significant predictors are in boldface.

surements (SD_i/SD_p) of the three traits (activity-aggression: $r_S = 0.08$, 95% CI = −0.15 to 0.33, $p = 0.357$; aggression-boldness: $r_S = 0.11$, 95% CI = −0.13 to 0.33, $p = 0.356$; activity-boldness: $r_S = -0.01$, 95% CI = −0.24 to 0.22, $p = 0.929$). Linear models were used to analyse the effects of infection intensities on the individual consistency of fish behaviours, while controlling for fish sex and size. The consistency of activity was associated with parasitic infection, being significantly negatively correlated with *Apatemon* sp. intensity and non-significantly but negatively correlated with both *Telogaster opisthorchis* and *Deretrema philippae* intensity (Table 2). Thus, fish with fewer *T. opisthorchis*, *D. philippae* and/or *Apatemon* sp. tended to show more consistent activity responses. Aggression was also significantly correlated with intensity of *T. opisthorchis* (Table 2), with greater infection associated with an increase in the consistency of aggression levels within individuals. There was no statistically significant effect from infection intensities on the consistency of boldness levels, while body size significantly and positively predicted the consistency of boldness scores, which was also true for activity.

4. Discussion

Although behavioural syndromes are characterized by consistent and repeatable differences among individuals, they are also subject to contextual influences, such as the perceived risk of predation (Bell & Sih, 2007; Dingemanse et al., 2007; present study). Parasitic infections may represent another important external factor acting on behavioural syndromes. Here, we have shown not only that parasitism is linked to specific behavioural traits in the common bully, *Gobiomorphus cotidianus*, but also that different parasite species are related in different ways to the consistency of behavioural responses displayed by individual fish. These findings join a small but growing body of evidence suggesting that parasitism can modify animal personality (Barber & Dingemanse, 2010; Coats et al., 2010). We acknowledge that our study is correlative in nature, and did not use controlled infections, which limits our inference. For instance, the direction of causality may be reversed, such that more active and exploratory fish might have acquired more infections by skin-penetrating trematodes (Wilson et al., 1993). We thus interpret our findings in light of this explicit caution.

Parasitic infection was not significantly related with activity or boldness of bullies, but greater infection by *Apatemon* sp. significantly correlated with aggression levels. Other factors also influenced individual behavioural traits. In session two, when a predator odour was present, both activity and aggression increased, while in session three, all behavioural responses increased. Furthermore, there was a significant increase in activity and boldness scores on the second day of all sessions. Fish body size correlated negatively with both activity and aggression levels. However, fish body size also correlated positively with infection levels by all parasites, probably a reflection of accumulation of parasites with age; the analyses we used, however, make it possible to disentangle the effects of size from those of infection. Overall, these results provide some support for our predictions that parasitic infection and perceived predation risk would alter behaviour.

Alterations of host behaviour induced by parasites may be a result of direct manipulation by parasites to increase their likelihood of transmission via predation, but they may also be a consequence of pathological side-effects from infection with little adaptive benefit for the host or parasite (Poulin & Thomas, 1999). Increased aggression associated with *Apatemon* sp. infection may have significant consequences for fish hosts, as dominant or more aggressive individuals are likely to have better access to food or mates (Kortet et al., 2010). Animals exhibiting high aggression levels however, may be more conspicuous to predators, potentially resulting in increased predation (Jakobsson et al., 1995; Sih et al., 2004; Stamps, 2007; Kortet et al., 2010). This could facilitate *Apatemon*'s transmission to its avian definitive host, though possibly also result in attacks by non-avian predators. This consequence of increased aggression may not apply equally to the other trematodes using bullies as intermediate hosts, *Stegodexamene anguillae* and *Telogaster opisthorchis*, since their eel definitive hosts are nocturnal predators locating prey by scent. An earlier study also showed little effect on aggression levels from *T. opisthorchis* in a closely related species, the upland bully *Gobiomorphus breviceps* (Hamilton & Poulin, 1995). However, the abundance of *T. opisthorchis* and *S. anguillae* was much lower than that of *Apatemon* sp.; thus, the latter may mask any potential effects from the other species (Poulin & Thomas, 1999).

Increased aggressiveness has been found in other species as a consequence of parasitic infection (Pontier et al., 1998; Mikheev et al., 2010). For instance, rainbow trout (*Oncorhynchus mykiss*) showed significantly increased aggression levels when infected with the eye fluke *Diplostomum spathaceum*, a trematode that also requires a definitive bird host (Mikheev et al., 2010). *Apatemon* sp. encyst in the muscle tissue and body cavity of fish, and until we elucidate the precise mechanism underlying altered aggression, simple pathology remains an alternative explanation to host manipulation (Poulin, 2010). Also, without experimental infection, it remains possible that aggressive fish acquire more parasites, rather than parasite infection making fish more aggressive. Indeed, aggressive individuals are more likely to acquire injuries from fighting with conspecifics, and this has been linked with a higher risk of parasitism in other systems (Glass et al., 1988; Pontier et al., 1998; Natoli et al., 2005). As *Apatemon* sp., *S. anguillae* and *T. opisthorchis* infect bullies by skin penetration, areas that have sustained damage may make this transmission route easier.

Although there were no direct links between parasitic infection and the other behaviours, activity and aggression were significantly increased in the presence of a predator cue (eel odour). This could presumably increase predation risk (Dingemanse et al., 2007). The high abundance of *Apatemon* sp. and other trematodes among our experimental fish makes it at least possible that parasites are responsible for suppressing anti-predator responses. However, there were also high scores of activity, aggression and boldness in session three, suggesting that the fish may have become conditioned to the behavioural tests, and less likely to exhibit anti-predator responses (Martin & Réale, 2008; Benesh et al., 2008). Acclimation to trial conditions, however, is unlikely to be the main explanation, as the effect on behaviour in session two was larger than the increase in session three.

Our results support the existence of correlations between traits in individual bullies within the population. There was a significant positive relationship between activity and aggression that was consistent in the presence and absence of predator odours, and over time. This means that more active individuals also tend to be more aggressive. Another relationship, between aggression and boldness, was only significant in the presence of a predator odour, with more aggressive fish also showing increased boldness. Because aggression was significantly associated with infection of *Apatemon* sp., the correlation between activity and aggression may be driven in part by an effect of parasitism. Although correlations that are persistent in populations are generally seen as beneficial to host organisms, they may actually be a consequence of parasitic infection (Barber & Dingemanse, 2010).

The relationship between aggression and boldness only became apparent in the presence of a predator odour, a pattern also found in sticklebacks (Bell & Sih, 2007). An aggression-boldness correlation has been found in several species of animals, including fish (Huntingford, 1976; Bell & Sih, 2007) and appears to be a common trait correlation. Both increased aggression and boldness may result in the increased likelihood of predation for fish, as they increase conspicuousness. Here, this correlation was only exhibited under perceived risk of predation. Exposure to eel odour might interact with infection by *Stegodexamene anguillae* and *Telogaster opisthorchis* to increase behaviours that may result in increased transmission. Again, elucidating the physiological mechanism responsible for the context-dependent correlation between aggression and boldness would be necessary to evaluate this possibility further.

Low intra-individual and relatively high inter-individual variation in behavioural responses is a basic characteristic of personality and behavioural syndromes (Dingemanse et al., 2007; Kortet et al., 2010). In our study, the overall repeatability estimates for all behaviours was low to moderate, with both activity and aggression showing higher repeatability compared to boldness. This, however, is likely a consequence of the alteration of behaviours during session two when the predator odour was present, when all behaviours were found to have very low repeatability. The low repeatability for all behaviours under perceived predation risk may be an adaptive response of the fish. An animal that exhibits decreased predictability may reduce its risk of predation, as a predator will be less likely to anticipate its movement and behaviour.

This would appear to be the aspect of behavioural syndromes that, if disrupted by infection, could potentially benefit trophically-transmitted parasites the most. Our most intriguing finding concerns the consistency of behaviours at the individual fish level. The consistency of activity was most associated with parasitic infection, as fish with fewer *Telogaster opisthorchis* and/or *Deretrema philippae* and *Apatemon* sp. exhibited higher consistency in their activity scores. Aggression was also associated with infection by *T. opisthorchis*: fish with more severe infections had more consistency in aggression levels. There also was no statistically significant correlation present between the consistency estimates of the three behavioural traits. The alteration of consistencies in behaviours such as activity and aggression may disrupt the dynamics of normally consistent behaviours, possibly resulting in increased predation on fish and increased transmission of the parasite (Barber & Dingemanse, 2010; Coats et al., 2010; Poulin, 2010). However, we have no evidence that altered behavioural consistency of the host affects transmission success of the parasites; these effects could merely be a pathological consequence of infection (Poulin, 2010); the loss of consistency in activity with increasing infections supports this view of pathology as an explanation. In systems where parasites are abundant, parasite-induced changes in behavioural consistency, whether or not they benefit the parasite, may have evolutionary implications for the host. Parasites are known to not only alter the mean value for behavioural responses, but also their variance (Poulin & Thomas, 1999). Through parasite-mediated selection on personality traits, parasites might contribute to the maintenance of genetic variation within host populations (Kortet et al., 2010).

Our findings reinforce the growing evidence that parasitism might influence host behavioural syndromes, with consequences for both hosts and parasites (Wilson et al., 1993; Barber & Dingemanse, 2010; Coats et al., 2010; Kortet et al., 2010; Poulin, 2010). On the one hand, behavioural syndromes may be ideal targets for adaptive host manipulation by parasites, as they influence predation risk and, thus, parasite transmission success. On the other hand, even if through plain pathology, parasites can act as external agents causing variation in behavioural syndromes, by affecting the consistency of behaviours or the correlations among behaviours. Alternatively, because our findings are based on correlations and not experimental infections, it is also possible that host behaviour determines the rates at which parasites are acquired, with behavioural syndromes being the cause of infection patterns and not their consequence. The next breakthrough may require the elucidation of the underlying physiological mechanisms linking infection with host behaviour, in order to distinguish between adaptation and side-effects. Clearly, more studies on this topic are required to better understand the link between parasitism and behavioural syndromes.

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