Original Article

Parasite infection in a central sensory organ of fish does not affect host personality

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Received 8 February 2016; revised 28 April 2016; accepted 30 April 2016; Advance Access publication 26 May 2016.

Among the ecological factors acting on the evolution and expression of animal personalities and behavioral syndromes, parasitism has received comparatively little attention. However, infection and host behavior are often strongly intertwined, because host behavior can predict the risk of infection and can also be changed by an infection. We conducted a controlled experimental infection study to explore the effects of infection on host boldness, exploration and activity using rainbow trout Oncorhynchus mykiss and its trematode parasite Diplostomum pseudospathaceum, which infects a central sensory organ of the fish, the eye lens. Contrary to our expectation, infection did not change the within-individual consistency of the personality traits or the relationships between the traits, but infected individuals were less bold than uninfected control individuals. We also found no relationship between personality in uninfected fish and resistance against parasite infection, suggesting that behaviors which increase parasite encounter do not select for improved resistance. Thus, we found only weak support for parasite-mediated effects on animal personalities.

Key words: animal personality, behavioral syndromes, host manipulation, parasites, resistance.

INTRODUCTION

Given the benefits of behavioral plasticity, recent research has focused on understanding why seemingly less flexible, consistent individual behaviors (personality traits), as well as behavioral correlations (syndromes) exist and how they are maintained (e.g., Sih et al. 2012; Niemela et al. 2013). Some studies have suggested that animal personalities and behavioral syndromes evolve as adaptive responses to local environmental conditions, such as predation pressure and food availability (e.g., Dingemanse et al. 2004; Bell and Sih 2007; Dingemanse et al. 2007). Among the other factors, parasitism is also known to influence the evolution of animal behaviors (Moore 2002), but its role in the evolution of host personality has only recently been acknowledged (Barber and Dingemanse 2010; Kortet et al. 2010). Essentially, parasitism could affect animal personality in 2 ways. First, selection may act on personality types or syndromes if the risk of acquiring infections depends on an individual’s behavior (Barber and Dingemanse 2010; Kortet et al. 2010). Here, behaviors related to key personality axes, such as shyness-boldness, exploration-avoidance and activity are particularly expected to affect parasite encounter rates (Barber and Dingemanse 2010). However, it is yet unclear whether different behavioral types similarly vary in resistance to parasites, or whether behaviors that increase parasite encounter are associated with increased resistance (Barber and Dingemanse 2010; Kortet et al. 2010).

Second, infection often leads to changes in host behavior because of pathological side-effects or direct manipulation of the host, which facilitates parasite development as well as transmission (Poulin 2010). For example, parasites that move between hosts by trophic transmission during their life cycle, have been shown to first enhance their intermediate hosts’ antipredatory behavior to secure time for development, and then to reduce it once infectivity and transmission capability to the next host is reached (reviewed in Parker et al. 2009). Consequently, infections may change the consistency of personality traits. Further, behavioral syndromes are known to evolve in response to predation risk (Bell and Sih 2007; Dingemanse et al. 2007). Thus, by decoupling such syndromes, trophically transmitted parasites could modulate host susceptibility to predation (Barber and Dingemanse 2010; Poulin 2010). However, very few empirical studies have considered the effects of parasitism on the stability of personality traits and behavioral syndromes (Coats et al. 2010; Hammond-Tooke et al. 2012; Kekäläinen et al. 2014). To our knowledge, only 1 study has used experimentally infected animals to exclude heterogeneities in infection history (Kekäläinen et al. 2014) and showed that infection affects personality and not vice versa (Poulin 2013).
Here, we studied personality traits and their correlations in rainbow trout *Oncorhynchus mykiss*, experimentally infected with the trematode eye fluke *Diplostomum pseudospathaceum*. This parasite has a complex life cycle involving 3 hosts: aquatic snails, fish and fish eating birds (Chappell et al. 1994). In fish, the parasite settles in the eye lens, where it damages the lens structure, induces cataracts and consequently impairs host vision (Karvonen et al. 2004a), an important sensory modality involved in fish behavior (Guthrie 1906). With increasing coverage of the eyes with cataracts, the susceptibility to predation also increases, suggesting that cataracts facilitate host manipulation in this system (Seppälä et al. 2005a). However, the parasite likely also manipulates its host before cataract formation, that is, soon after infection when it is not yet ready for transmission to the final host, by enhancing host anti-predatory behavior (Gopko et al. 2015). We estimated boldness, exploration and activity—3 behaviors of fish that may affect susceptibility to predation—before infection, 16 days post infection (parasite not yet infective to final host) and 38 days post infection (parasite infective to final host) and tested whether infection changes the temporal consistency of personality traits and decouples behavioral syndromes. Further, we explored whether behavior 38 days post infection is related to cataract size and whether there is a link between personality traits expressed in uninfected individuals and their resistance against *D. pseudospathaceum*.

**METHODS**

**Host and parasite sources**

One-year-old rainbow trout were obtained from a ground-water-using fish farm, which ensured that fish were free of infections. Ten days before the experiments commenced, 100 fish were randomly distributed among 5 groups of 20 individuals and individually marked with visible implant elastomer tags (Northwest Marine Technologies, Shaw Island, Washington). The fish were maintained in these groups throughout the experiment in five 170 L holding tanks, among which they were shuffled following each personality test (see below) and were fed daily with commercial fish pellets.

Parasites used for the experiments originated from 10 *Lymnaea stagnalis* snails naturally infected with *D. pseudospathaceum*. The snails were collected from Lake Vuojärvi (Central Finland) 1 week before the experimental exposures and kept at 4 °C in individual containers with 1 L of lake water and lettuce *ad libitum*.

The experiment was carried out with permission from the Finnish Regional State Administrative Agency (license no. ESAVI/6367/04.10.03/2011) and complied with the animal care legislation of Finland.

**Experimental exposure**

Parasite cercariae were obtained from the snails by placing them individually in 200 mL of lake water (17 °C). After 4 h, the suspensions from all snails were combined and parasite density was estimated from ten 1 mL samples. All fish were placed individually in round containers with 2 L of lake water (17 °C). Ten fish of each group were exposed to 250 parasites whereas the other half was sham exposed with lake water. The exposure dose was chosen to obtain infection intensities that resemble those of wild fish, which typically range between 0 to 100 (Valtonen and Gibson 1997; Marcogliese et al. 2001; Karvonen and Seppälä 2008) and can be up to several hundred in rainbow trout (Wootten 1974). After 30 min of exposure, all fish were returned to their holding tanks.

**Estimation of personality traits**

Boldness, explorative behavior and activity were assessed in 3 rounds: before the exposure, 16 days post exposure and 38 days post exposure (methodology modified from earlier fish personality studies, e.g., Kekäläinen et al. 2014; Kortet et al. 2014; Naslund et al. 2015). Fish were first individually placed into a closed refuge within an otherwise illuminated (800 lux) tank (120 × 20 × 20 cm, Figure 1) made of green plastic and containing 24 L of lake water (17 °C). After 5 min of acclimation, a divider that restricted fish to the refuge was gently removed, and the latency until complete emergence of fish from the refuge was measured. Thus, boldness was assessed as response to a novel environment with shorter latency to emerge indicating greater boldness. Following emergence, fish were observed for explorative behavior. For this, the tank outside the refuge was divided into 5 equally sized zones (Figure 1), and the number of zone crosses within 10 min after emergence was counted. Thus, exploration was measured as space use in a novel environment. Finally, after habituation to the test tank, which typically takes about 2 h for rainbow trout (Mikheev et al. 2013), activity was assessed as the total time spent moving during 20 min starting 2.5 h after opening the refuge. Following this, all fish were identified and transferred back to their holding tanks.

The experiment was conducted using 10 identical tanks, simultaneously testing 10 fish of the same group. The water was changed after each test. Fish behavior was recorded using video cameras above the tanks and the recordings were scored for the behavioral traits by 1 observer blind to infection treatment.

Finally, all fish were euthanized with an overdose of MS-222 and measured for length (control: 170.5 ± 16.1 standard error [SE] mm, infected: 166.4 ± 14.4 mm; t9 = −1.194, P = 0.236). Both eyes were investigated using a slit-lamp microscope to score the lens areas covered with cataracts to the nearest 10% (Karvonen et al. 2004a). Eye lenses were then dissected for parasite numbers and the parasite

**Figure 1**

Test tank for the estimation of fish boldness (latency to emerge from shelter after the divider was removed), exploration (the number of grid marks fully crossed during 10 min after emergence) and activity (the time in seconds spend moving during 20 min after 2.5 h habituation in the tank).
sum of the right and left lens was used as an inverse measure of resistance against the infection. All sham-exposed fish remained uninfected, whereas all parasite exposed fish became infected.

Statistics
Due to mortality, loss of ID tags and 2 individuals not exiting the refuge in the third round, the final sample size was 38 fish (40 infected) in the first and second round and 76 (34 infected) in the third round. All personality data were square root transformed for normality. Repeatability of the traits among the rounds was analyzed as intraclass correlation coefficient (±95% CI) separately for control and infected fish in SPSS (IBM Statistics v. 22). Relationships between the traits were analyzed using mixed models in SAS (v. 9.4) separately for infected and control fish with experience as random factor and fish ID as repeated factor. Between-individual correlations were extracted as suggested by Dingemanse and Dochtermann (2013) and the significance of the correlations was determined using likelihood ratio tests. Effects of round (1–3), infection and fish length on the expression of personality traits was analyzed by fitting repeated mixed models in SAS, again using experimental group as random factor and fish ID as repeated random factor. Finally, the effects of cataract size on personality traits (third round), and of personality traits (first round) on parasite number (inverse resistance) were explored using Spearman’s rank correlations.

RESULTS
All personality traits were significantly repeatable, except for explorative behavior in control individuals, which was close to being significant (Table 1). Among control fish, there was a significant negative correlation between latency to emerge from the refuge and activity (Table 2, Figure 2), suggesting higher activity in bolder individuals. Further, there was a positive relationship between exploration and activity, but no significant correlation between boldness and exploration (Table 2, Figure 2). In contrast, none of the behaviors were significantly related among the infected individuals, although the relationships between boldness and activity, as well as exploration were also positive and close to significance (Table 2, Figure 2). It is important to note that the exploration—activity relationship was generally weak in this group, that is, also before infection (Figure 2).

Boldness was significantly affected by an interaction between infection status and round ($F_{2,222} = 3.61$, $P = 0.029$). This was because boldness of control fish changed over time (Post hoc pairwise comparisons of least-square means: $b_{1.149} = 9.99$, $P < 0.001$), while it stayed stable for infected fish ($b_{2.149} = 0.0$, $P = 0.905$), resulting in significantly bolder control fish than infected fish in the third round ($b_{1.149} = 7.15$, $P = 0.008$, Figure 3). Boldness was not affected by fish length ($F_{2,222} < 0.01$, $P = 0.975$). Explorative behavior and activity differed between the rounds, so that fish became more explorative ($F_{2,222} = 6.02$, $P = 0.003$) but less active ($F_{2,222} = 13.36$, $P < 0.001$) with experience (Figure 3). However, these traits were not affected by infection ($F_{2,222} = 1.80$, $P = 0.181$ and $F_{2,222} = 0.32$, $P = 0.575$, respectively). Neither the interaction terms nor length were significant (all $P > 0.083$).

Among infected fish, the mean area of eye lenses covered by cataracts was $86.1 \pm 2.6\%$ (range 30–100%). Cataract coverage did not affect the expression of personality traits 38 days post infection ($N = 34$, latency to emerge $r_s = 0.216$, $P = 0.219$, exploration $r_s = -0.241$, $P = 0.170$ and activity $r_s = -0.319$, $P = 0.066$). The average parasite number per fish was $50.3 \pm 2.9$ with a range of 9–111. There was no relationship between the personality traits before infection and the number of parasites established during infection, that is, inverse resistance ($r_s = 0.185$, $P = 0.254$, exploration $r_s = -0.016$, $P = 0.922$ and activity $r_s = -0.102$, $P = 0.531$).

DISCUSSION
Due to their interplay with host behavior, parasites may affect the evolution and expression of personality traits and behavioral syndromes (Barber and Dingemanse 2010; Kortet et al. 2010). We followed personality traits in rainbow trout from the uninfected to the infected stage, and in the latter during parasite development, which is linked to its infectivity to the final predatory host. In contrast to our prediction, we found no evidence of infection with D. pseudospathaceum affecting the consistency of host personality traits. This result is surprising, as this parasite has been noted for its significant effects on host behavior due to the site of infection in a central sensory organ, the eye lens. For example, experimental studies using comparable infection intensities to this study have demonstrated that infected rainbow trout show reduced escape behavior (Seppälä et al. 2004), are more vulnerable to simulated predation (Seppälä et al. 2004, 2005b), reduce cryptic behavior (Seppälä et al. 2005a) and undergo changes in aggression levels during parasite development (Mikheev et al. 2010). These behavioral changes are attributed to the effects of the parasite on host vision and thus, suggest parasite manipulation (Seppälä et al. 2005b). Given the high average parasite number and cataract coverage in the fish, the present results suggest that the stability of the personality traits is unaffected by infection. Trait stability may instead be subject to inherited genetic factors, suggested by the general finding that personality traits are heritable (e.g., van Oers et al. 2004; Dingemanse et al. 2009; Kortet et al. 2014).

Despite the consistency of behavioral traits throughout this study, however, infected individuals were less bold than control fish when parasites were fully developed (38 days post infection). This is in contrast to findings of a previous study on 2 natural populations of Eurasian minnows (Phoxinus phoxinus), one experiencing high parasitism and predation risk and showing higher boldness compared with the other parasite and predator free population (Kortet et al. 2015). In the present study, the difference in boldness may be due to interventions of the parasite whereas in the natural populations, the increase in boldness may be due to increased risk factors from the parasites and predators.
explained by pathological side-effects of infection, for example, if energetic costs of parasitism affect the cost-benefit ratio of boldness (Poulin 2013). Alternatively, this may suggest an adaptive host response to enhance predator avoidance (Barber and Dingemanse 2010) if reduction in fish vision by cataracts makes risk assessment more difficult. This explanation seems very probable, because boldness of control fish decreased in repeated tests, suggesting habituation to the test situation, whereas boldness of infected fish was constant throughout the experiment. However, there was no indication that the degree of host vision impairment would be related to boldness. From the parasite’s perspective, reduced risk taking of an intermediate host seems beneficial. It is possible, however, that specific manipulations of a single personality axis may decouple behavioral correlations (Barber and Dingemanse 2010), such as those involved in antipredator strategies. For example, behavioral correlations may be involved in fish shoaling behavior, a tendency for which is exhibited by shy individuals that rely on group protection, whereas bold individuals are more solitary and may rely on risk assessment through predator inspection (e.g., Budaev 1997; Ward et al. 2004). Indeed, earlier research has shown that infection with D. pseudospathaceum decreases fish shoaling behavior (Seppälä et al. 2008), which together with lower levels of boldness may result

Table 2
Between-individual correlations for 3 personality traits estimated 3 times in infected and control fish

<table>
<thead>
<tr>
<th>Boldness–Exploration</th>
<th>Boldness–Activity</th>
<th>Exploration–Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>$X^2$</td>
<td>$P$</td>
</tr>
<tr>
<td>Control</td>
<td>−0.640</td>
<td>2.9</td>
</tr>
<tr>
<td>Infected</td>
<td>−0.632</td>
<td>2.8</td>
</tr>
<tr>
<td>Controls</td>
<td>−0.947</td>
<td>6.3</td>
</tr>
<tr>
<td>Infected</td>
<td>−0.689</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>0.987</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>0.398</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Significance values were calculated using likelihood ratio tests, $df = 1$.

Figure 2
Relationships between latency to emerge and exploration (a, b), latency to emerge and activity (c, d) and exploration and activity (e, f) for control (a, c, e) and infected individuals (b, d, f). Note that all values have been square-root transformed and that linear regression lines have been fitted only for illustration, whereas between-individual correlation coefficients have been calculated for statistical verification of relationships (Table 2).
in a net increase in host susceptibility to predation and enhance parasite transmission.

Nevertheless, the behavioral correlations studied here were not affected by infection. Although these associations appeared to be weaker among infected compared with control fish, this difference was already evident before the infections (Figure 2). Coats et al. (2010) demonstrated stronger behavioral correlations in amphipods infected with Microphallus sp. compared with healthy conspecifics, but individuals in this study were naturally infected which does not exclude the possibility that personality affected infection patterns. The only experimental infection study looking at the stability of behavioral syndromes so far demonstrated minor effects of infection: Eurasian minnows (P. phoxinus) heavily infected with the brain parasite Diplostomum phoxini showed weaker positive associations between personality traits than healthy individuals or individuals with low infection intensities, whereas the difference between these correlations was not significant (Kekalainen et al. 2014). Thus, the current evidence does not strongly support the idea that syndromes instead of behavioral traits could be the target of host manipulation (Poulin 2010).

The different lifestyles of individuals at both ends of key personality axes likely affect parasite exposure with more bold, explorative and active individuals facing higher encounter rates (Barber and Dingemanse 2010). A recent study on a tadpole-trematode host-parasite system demonstrated negative relationships between activity, as well as explorative behavior and susceptibility to infection (Koprivnikar et al. 2012). However, the authors suggested that susceptibility was not related to behavior per se, but that more active and explorative individuals likely possess more effective anti-parasite behaviors or are better able to resist the infection. Indeed, Kortet et al. (2007) demonstrated a positive link between boldness and immunocompetence in field crickets (Gryllus integer), suggesting that animals are able to compensate for behaviors that increase the likelihood of parasite encounter. Here, we found no relationship between the personality traits in uninfected individuals and resistance against subsequent infections. However, rainbow trout show effective anti-parasite behaviors by moving away from areas of risk on parasite encounter (Karvonen et al. 2004b), which is why behaviors that increase parasite exposure may not select for increased resistance.

Overall, our study does not support an effect of parasitism on the consistency of animal personality traits or their correlations. However, infected individuals were less bold than uninfected control individuals, the underlying causes of which require further work. Together with the results of Kekalainen et al. (2014), this work suggests that manipulative effects of parasites on animal personalities, if any, are probably weak, at least under laboratory conditions.

**FUNDING**

This work was funded by the Academy of Finland grants #263864 and #292736 to A.K.

We would like to thank Komnevesi research station for research facilities and support in fish maintenance. We also acknowledge 2 anonymous reviewers for their helpful comments. Data accessibility: Analyses reported in this article can be reproduced using the data provided by Klemme et al. (2016).

**Handling editor:** John Fitzpatrick

**REFERENCES**


