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Potential multidimensional behavioural impacts of differential infection in two fish populations

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Abstract

Parasites can influence the behaviour of their hosts, however investigations on how they may shape multiple personality traits are uncommon. The flatworm parasite *Tylodelphys darbyi* resides in the eyes of common bully, *Gobiomorphus cotidianus*, a locally common fish host to a range of other parasites that could also influence their behaviour. Here we assess how parasitism may be related to personality traits of two *G. cotidianus* populations; one where *T. darbyi* is highly abundant and one where the parasite is absent. We hypothesized that *T. darbyi* would have a stronger association with the different personality traits than other parasite taxa, and that the effects of infection on personality traits would vary between populations. Our results demonstrate that *T. darbyi* infections correlate with boldness, exploration, and activity within and among individuals. Further, we show that the relationship and therefore possible influence of other parasites, e.g., *Apatemon* sp., on personality traits vary between two host populations. Our study has revealed potential patterns highlighting how parasitism may differentially contribute to behavioural and ecological divergence among host populations.

Keywords

eye-flukes, personality traits, host-parasite interactions, *Gobiomorphus cotidianus*, *Tylodelphys darbyi*.

1. Introduction

It is well established that diseases caused by parasitic organisms can influence the behaviour of their host, but studies on how they may shape personality traits are relatively recent (Barber & Dingemanse, 2010; Kortet et al., 2010; Hammond-Tooke et al., 2012; Klemme et al., 2016; Barber et

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al., 2017). Personality traits can vary among individuals within a population while remaining consistent and repeatable within individuals, creating distinct personalities (Sih et al., 2004a, b; Dingemanse & Réale, 2005; Réale et al., 2007). These traits, such as boldness, exploration, and aggression, can correlate with one another, forming behavioural syndromes, which can shape population dynamics, resource acquisition, and social interactions (Réale et al., 2007; Barber & Dingemanse, 2010; Wolf & Weissing, 2012). Parasitism can interact with personality in at least two ways, reflecting two alternative chains of causation: either an individual's personality affects its exposure and susceptibility to parasite infection, or infection by parasites induce changes in the host's personality, or both (Poulin, 2013; Klemme et al., 2016). Some personality types can make individuals more likely to become infected than others. In the aquatic environment in particular, the most explorative and bold individuals in a population could be more likely to encounter infective stages simply due to their movement about the environment (Poulin et al., 1991; Wilson et al., 1993; Kortet et al., 2010; Koprivnikar et al., 2012). Conversely, parasites can alter the behaviour of their host through several pathways, including pathological side-effects and direct manipulation to facilitate completion of the parasite's lifecycle (Poulin, 2010).

Most studies on parasite-induced behavioural changes have historically focused on a single personality trait rather than multiple axes (Barber et al., 2017). For example, studies relating infection to a fish's ability to pursue mates due to physical impairment, or increased predation rates as a result of conspicuous swimming patterns, include activity level while not specifically referring to it as a personality trait (Lafferty & Morris, 1996; Cureton II et al., 2011; Fredensborg & Longoria, 2012). However, there have been recent efforts to take a multidimensional approach, in order to demonstrate that parasite infection is associated with multiple personality traits (Cézilly & Perrot-Minnot, 2005; Thomas et al., 2010; Cézilly et al., 2013; Kekäläinen et al., 2014; Klemme et al., 2016). This approach has revealed that parasitic infection can result in multiple behaviours being altered, potentially, in some cases, increasing the host's risk of predation (Gopko et al., 2017). In addition, some parasites may influence the host by reducing the consistency of, rather than directly altering, particular behaviours (Coats et al., 2010; Hammond-Tooke et al., 2012; Poulin, 2013).

Certain groups of parasites, such as those that invade the nervous system or sense organs, are more likely to influence personality traits than others (Barber & Crompton, 1997a, b; Barber & Wright, 2006). Diplostomid trematodes commonly infect the eyes of fish as part of their life cycles where, depending on the species, they can inhabit the lens, humours, or retina. *Diplostomum* spp., which reside in the lens, are known to induce cataract formation in their hosts (e.g., rainbow trout *Oncorhynchus mykiss*, cyprinids, etc.) whereby they can impair a variety of visually sensitive behaviours (Karvonen et al., 2004; Seppälä et al., 2004, 2005, 2008, 2011). For instance, infected trout when threatened by a predator tend to be more active, quicker to recover, form less cohesive shoals, and in general are more likely to be captured than uninfected fish (Seppälä et al., 2004, 2008; Gopko et al., 2017). It has also been shown that *D. spathaceum* infected *O. mykiss* are more aggressive yet lose territorial contests more often than uninfected fish (Mikheev et al., 2010). Surprisingly, Klemme et al. (2016) found *D. pseudospathaceum* did not impact personality traits (i.e., boldness, exploration, and activity) in *O. mykiss* compared to uninfected fish under experimental conditions (Klemme et al., 2016). These findings contrast with those by Gopko et al. (2017) that found infected fish were more active and bolder than uninfected conspecifics, suggesting that host-parasite interactions may differ among congeneric parasites and should be observed in multiple settings.

Another group of diplostomids, *Tylodelphys* spp., can reside in the humours of the eyes and occur worldwide. Some species can impair the ability of their fish hosts to identify and acquire prey, especially in competition with uninfected individuals (Muñoz et al., 2017, 2019). In New Zealand, *Tylodelphys darbyi* resides in the aqueous and vitreous humours of common bully *Gobiomorphus cotidianus*, a native fish widespread in rivers and lakes. Metacercariae of this species can reach a large size (about 1 mm long by 0.4 mm wide), are mobile and can occur in large numbers (>15 per eye) in infected fish (Blasco-Costa et al., 2017). Recent work has demonstrated that *T. darbyi* may have some impact of microhabitat choice and activity level of *G. cotidianus* while not impairing their ability to recognize and avoid a simulated predator (Ruehle & Poulin, 2019, 2020). However, both studies focused on specific responses from the host rather than taking a multidimensional approach and investigating a suite of personality traits. So, it is possible that these eye-flukes have the potential to influence a full suite of personality traits and correlations among them.

Tylodelphys darbyi is restricted to mountain lakes on the South Island of New Zealand (unpublished data) whereas *G. cotidianus* is found throughout the country. Population differences in personality are already well established for other taxa, and the broad distribution of *G. cotidianus* means that different populations have unique parasite assemblages, potentially with different behavioural consequences (Bell & Sih, 2007; Dingemanse et al., 2007; Kortet et al., 2015). Hammond-Tooke et al. (2012) investigated the effects of several trematode species on personality traits of bullies from a coastal lagoon, Lake Waihola, where *T. darbyi* does not occur. In the present study, we compare two *G. cotidianus* populations, one with *T. darbyi* (i.e., Lake Hayes) and the other where the parasite is absent (i.e., Lake Waihola; Hammond-Tooke et al., 2012) yet both populations share the trematodes *Apatemon* sp., *Telogaster opisthorchis*, and *Stegodexamene anguillae* which encyst throughout the musculature and body cavities. We address the following questions: 1) is *T. darbyi* intensity associated with different personality traits in bullies? 2) how do other parasites in the Hayes population potentially influence host personality? and 3) are there population level differences in personality traits with respect to infection by similar parasites? We hypothesize that *T. darbyi*, given its location in the eye, will have greater potential to influence the different personality traits compared to other parasite taxa, and that the association between infection and personality traits will vary between populations.

2. Methods

2.1. Collection, housing and tagging

Common bullies were collected from Lakes Waihola ($N = 14$) and Hayes ($N = 31$) on the South Island of New Zealand in Summer 2019. Hayes is a small glacial lake located in the Southern Alps in the Otago Region. Fish at this site were collected using minnow traps placed along the littoral zone as the rocky bottom (e.g., gravel) combined with the benthic nature and sparseness of the bullies made seine netting impractical. In contrast, Waihola is a coastal, tidal lake 40 km from the university and the topography (e.g., silt) of the lakebed and high number of bullies allowed collection via seine. Both lakes possess nearshore structure in the form of woody debris, rocks, and aquatic vegetation used by *G. cotidianus* as shelter. All fish, regardless of collection site, were transported in 20-litre ice chests provided with aeration back to the university and transferred immediately to holding tanks (36 litres; $44 \times 27.5 \times 29.5$ cm). Bullies were held in size-matched groups, of no more than 8 fish, were provided with filtration, aeration, and fed ad libitum on commercial fish pellets daily. Holding tanks were filled with a solution of 1/3 saltwater and 2/3 freshwater to prevent fungal growth on fish, which can reach lethal levels in captivity without treatment. Fish were allowed 72 h acclimation to housing conditions (12 h day/night light cycle; 20°C water temperature) before being labelled with, under anaesthesia (5 min in a MS-222 solution, 1 mg/l), unique 2 mm subcutaneous elastomer tags (VIE) for individual identification (Northwest Marine Technology, Anacortes, WA, USA).

2.2. Personality traits

We tested 4 personality traits, boldness, exploration, activity, and aggression, in both populations of common bully. Experiments took place in three sessions, with 7 days of rest in between each, in which personality traits were measured successively and in the same order: exploration/activity, boldness, and aggression. Ten test arenas (8 litres; 36 × 16 × 14 cm), filled to a depth of 5 cm, were used for the experiments; each was equipped with a PVC tube (10 × 5 cm diameter) to act as shelter and had a 4 × 2 grid drawn (overall: 20 × 15 cm; each cell: 5 × 7.5 cm) on the bottom to assess a fish's movement. Bullies were tested alone in the arenas; an opaque covering ensured they could not see fish in adjacent tanks as well as minimized visibility of the observer. No fish was tested in the same arena in more than one session.

Fish were introduced to an arena at the end opposite the shelter and held there behind an opaque divider (14.7 × 9.5 cm) for 5 min to acclimate. Afterward the divider was gently removed, and the fish were filmed from above for 3 min. The video was assessed later and the time a fish spent moving during the 3 min was recorded as activity, whereas exploration was measured as the number of times a fish moved between grid cells. Once the filming period ended a lid was gently placed on the arena and the fish allowed 1 h of rest before being tested for the next trait. To assess boldness, we measured the latency to emerge from a shelter following a simulated predatory attack (modified from Hammond-Tooke et al., 2012). A metal rod (154 cm length) with a black, plastic square (16 × 10 cm) attached to the end was thrust through the arena, in a standardized way and always by the same observer, without impacting either the fish or the shelter. Doing so in this manner provides both visual and tactile (i.e., due to the moving water) stimuli for the fish. Following the predatory strike, the bullies were observed for 3 min and the time taken to emerge from the shelter was recorded. A bully was determined to have "emerged" if the entire head was visible to the observer. If a bully did not emerge by the end of the observation period, it was scored as taking longer than 3 min, and if they did not enter the shelter they received a score of 0 s. Finally, after another 1hr recovery period, aggression was measured as the amount of time a bully spent facing its reflection and performing darts toward or strikes against the mirror in a 3 min period (modified from Hammond-Tooke et al., 2012). A mirror (16 × 13 cm) was placed in the arena lengthwise with the opaque side (i.e., the back) facing out and reflective side against the wall for 5 min to acclimate the fish to a novel object. Following the acclimation period, the mirror was flipped around to reveal the reflective side and a further 30 s was allowed for the fish to adjust to the mirror before observation began. During the aggression test no fish struck the mirror with enough force to cause itself harm.

2.3. Parasite counts

Following the final session, fish were killed by an overdose of MS-222 (10 mg/l) and both standard length (SL; mm) and total mass (TM; g) were recorded. The eyes were removed and examined separately for *Tylodelphys darbyi* intensities in left and right eyes. Following assessment of the eyes, we dissected the rest of the fish to record any other parasites. The trematodes *Apatemon* sp., *Telogaster opisthorchis*, and *Stegodexamene anguillae* are commonly found encysted as metacercariae in the muscle tissues, connective tissues of the body cavity, and various organs in bullies from both populations. Fish from Waihola have been found to be infected by adults of *Coitocaecum parvum*, also a trematode, inside the digestive tract. A single larval nematode, *Eustrongylides* sp., is commonly reported from both lakes in small numbers within the body cavity. All of these were counted in each fish used in the experiments; counts of each parasite species were included as separate predictors in the statistical analyses.

2.4. Statistical analysis

Statistical tests were performed, and figures generated using the program R (version 3.6.1; R Core Development Team, 2019). Due to many fish not reacting in the aggression test or not emerging from the shelter during the boldness test, these data have an excess of 0 or >3 min (i.e., >180 s) values, respectively. Previous studies have transcribed such data into a binomial form in order to run standard generalized mixed models (e.g., Hammond-Tooke et al., 2012); however, this results in a loss of variation in the data. In order to account for the excess of extreme values and retain the variation in the data, we standardized the data recorded for each personality trait using the formula $\frac{(\frac{\text{(Sum of Behavioral Scores)}}{\text{(\# of Sessions)}})}{10 \div \text{Maximum Score}}$ such that each fish received a score of 0–10. For the timed behaviours, i.e., boldness, activity, and aggression, the maximum score was 180 s, but for exploration we took 100 crosses between sections to be the maximum as the highest score recorded was 97 (see Results). Standardization allows all personality traits to be examined on the same scale and reduces the impact of extreme values. In addition, Spearman's rank correlations between the four traits, untransformed, were calculated with 95% confidence intervals (CI) for all fish and within each population.

We ran 3 sets of linear models using the standardized scores of the personality traits as response variables. The first set of models looked specifically at the impact of *T. darbyi* on the traits, so Waihola fish were excluded as the parasite is not present in that population. Each trait was used as a response variable in individual models with *T. darbyi* intensity and sex of the fish as predictors. Standard length correlated strongly with *T. darbyi* intensity ($r^2 = 0.68$), and as such was removed to simplify the models. A second set of models aimed to address the impacts of parasitism in general on the personality traits within the Hayes population. So, again each trait was used as a separate response with intensities of *T. darbyi*, *Apatemon* sp., *T. opisthorchis*, *S. anguillae*, SL, and sex as predictors. The final set of models compared the impacts of the parasites shared between the populations, so *T. darbyi* was excluded from these. Predictor variables for these models were the two-way interactions between population and intensities of *Apatemon* sp., *T. opisthorchis*, *S. anguillae*, and SL as well as the main effect of population.

We calculated between and within individual repeatability estimates for each personality trait for both populations. For between individual values, we used the *rptR* package (Nakagawa & Schielzeth, 2010) to calculate estimates for all fish as well as for each population separately. Within individual repeatability was calculated by taking the ratio of individual-to-population standard deviation ($\text{SD}_\text{i}/\text{SD}_\text{p}$) (Réale & Dingemanse, 2010; Hammond-Tooke et al., 2012). We then ran 3 series of linear models (LM; *lm*) using the standard deviation ratio for each trait as the response. One set of models compared the consistency of behaviours between populations, so again *T. darbyi* was excluded and the two-way interactions between population and *Apatemon* sp., *T. opisthorchis*, *S. anguillae*, and SL as well as the main effect of population were used as predictors. The other two model series compared consistency within each population separately using each parasite species (except for *T. darbyi* in Waihola), SL, and sex as predictor variables.

3. Results

We recovered 5 parasite taxa from the Hayes and Waihola populations of *Gobiomorphus cotidianus* (Table 1). *Tylodelphys darbyi* is the only species not shared between the populations but was found in the humours of all fish sampled from Lake Hayes at relatively high intensities in some individuals. Of the shared taxa, 3 were trematodes, *Apatemon* sp., *Telogaster opisthorchis*, and *Stegodexamene anguillae*, and the other the nematode *Eustrongylides* sp. The Hayes population had higher overall intensities of *Apatemon* sp. while Waihola fish had greater infections of *T. opisthorchis*. *Eustrongylides* sp. and *S. anguillae* had similar intensities in both populations with the former being more prevalent in Hayes.

Table 1.
Percent prevalence, mean intensity, and range of intensity for all parasite taxa recovered for each population.

Population	Helminth taxa	Prevalence (%)	Mean intensity	Intensity range	
				Min	Max
Hayes ($N = 31$)					
	Tylodelphys darbyi	100.0	34.9	5	99
	Apatemon sp.	100.0	281.0	49	597
	Telogaster opisthorchis	64.8	4.3	0	12
	Stegodexamene anguillae	74.7	3.5	0	10
	Eustrongylides sp.	47.3	1.3	0	2
Waihola ($N = 14$)					
	Apatemon sp.	100.0	170.4	105	224
	Telogaster opisthorchis	100.0	11.4	1	21
	Stegodexamene anguillae	71.4	5.5	0	12
	Eustrongylides sp.	7.1	1.0	0	1

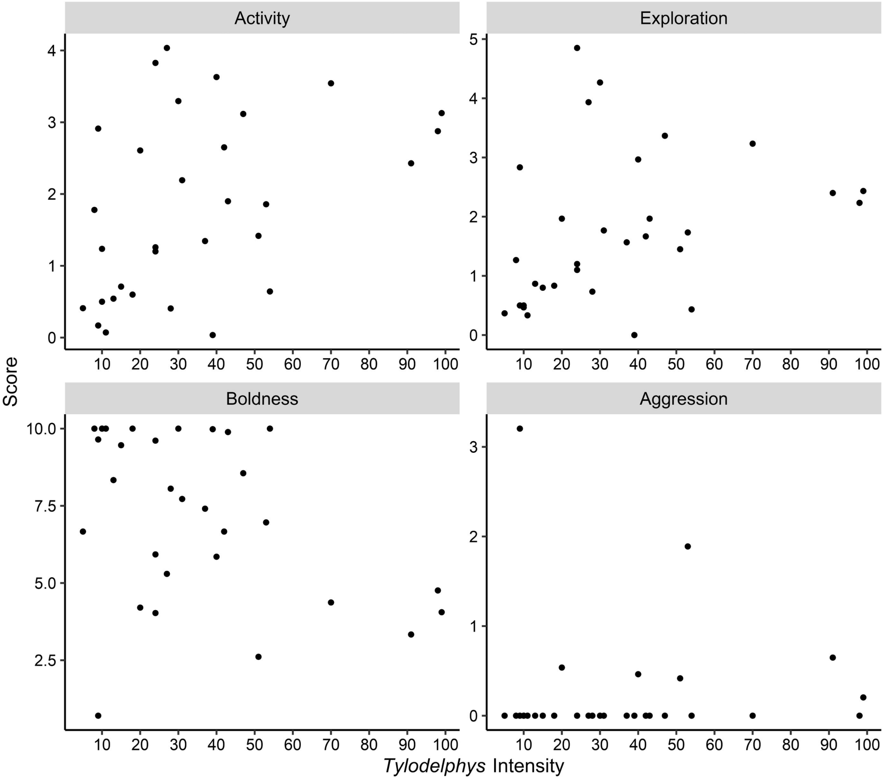

Figure 1. Scatterplots showing relationship between standardized scores for activity, exploration, boldness, and aggression of common bullies, *Gobiomorphus cotidianus* ($N = 31$), and *Tylodelphys* intensity in the Lake Hayes population. Relationships for activity, exploration, and boldness were significant ($p < 0.05$).

The first model series (Figure 1; Table 2) shows that *T. darbyi* on its own significantly predicts boldness ($t = -2.529$, df $= 28$, $p = 0.017$), activity ($t = 2.748$, df $= 28$, $p = 0.010$), and exploration ($t = -2.130$, df $= 28$, $p = 0.042$). In the Hayes population models considering all parasite species (Table 2), *T. darbyi* still predicts boldness ($t = -2.506$, df $= 24$, $p = 0.019$) but no other personality traits (Appendix A at 10.6084/m9.figshare.12860627). However, *Apatemon* sp. intensity significantly predicts exploration ($t = 2.270$, df $= 24$, $p = 0.032$) in the Hayes population model (Figure 2; Table 2). Likewise, in the models comparing the two populations, *Apatemon* sp. intensity predicts activity ($t = 2.270$, df $= 35$, $p = 0.29$) and exploration ($t = 2.445$, df $= 35$, $p = 0.020$) in Hayes but not in Waihola

Table 2.
Significant interactions ($p < 0.05$) of the three linear model series: effects of *Tylodelphys* only, effects of all parasites from Lake Hayes, and differences between the Hayes and Waihola populations.

	Predictors	Estimate	SE	t	p
Tylodelphys (df = 28)					
Boldness	Intercept	6.749	0.701	9.632	<0.001
	Tylodelphys	−0.045	0.018	−2.529	0.017
Exploration	Intercept	2.223	0.323	6.887	<0.001
	Tylodelphys	0.017	0.008	2.130	0.042
Activity	Intercept	2.136	0.315	6.779	<0.001
	Tylodelphys	0.022	0.008	2.748	0.010
Aggression		–	–	–	–
Hayes Parasites (df = 24)					
Boldness	Intercept	6.661	0.692	9.621	<0.001
	Tylodelphys	−0.071	0.028	−2.506	0.019
Exploration	Intercept	2.234	0.310	7.200	<0.001
	Apatemon	0.004	0.002	2.270	0.032
Activity	Intercept	2.133	0.293	7.277	<0.001
Aggression		–	–	–	–
Hayes vs Waihola (df = 35)					
Boldness	Intercept	6.657	0.841	7.918	<0.001
Exploration	Intercept	1.536	0.301	5.100	<0.001
	Apatemon: Hayes	0.003	0.001	2.445	0.020
Activity	Intercept	1.566	0.325	4.821	<0.001
	Apatemon: Hayes	0.003	0.002	2.270	0.029
Aggression	Intercept	0.405	0.168	2.412	0.021

See Appendix A at 10.6084/m9.figshare.12860627 for full results.

fish (Figure 2; Table 2). All other variables did not significantly predict any personality traits in either population (Appendix A at 10.6084/m9.figshare.12860627).

Spearman correlations found between behaviours for all fish as well as within populations are shown in Figure 3. These indicate that overall the most exploratory fish are also more active and that both behaviours have a positive relationship with aggression. Likewise, the fish that emerge from the shelter sooner (i.e., bold) also tend to be more aggressive overall. Within the Hayes population, all the patterns remain except that more active fish also emerge from the shelter sooner. However, in the Waihola fish only the relationship between exploration and activity remains.

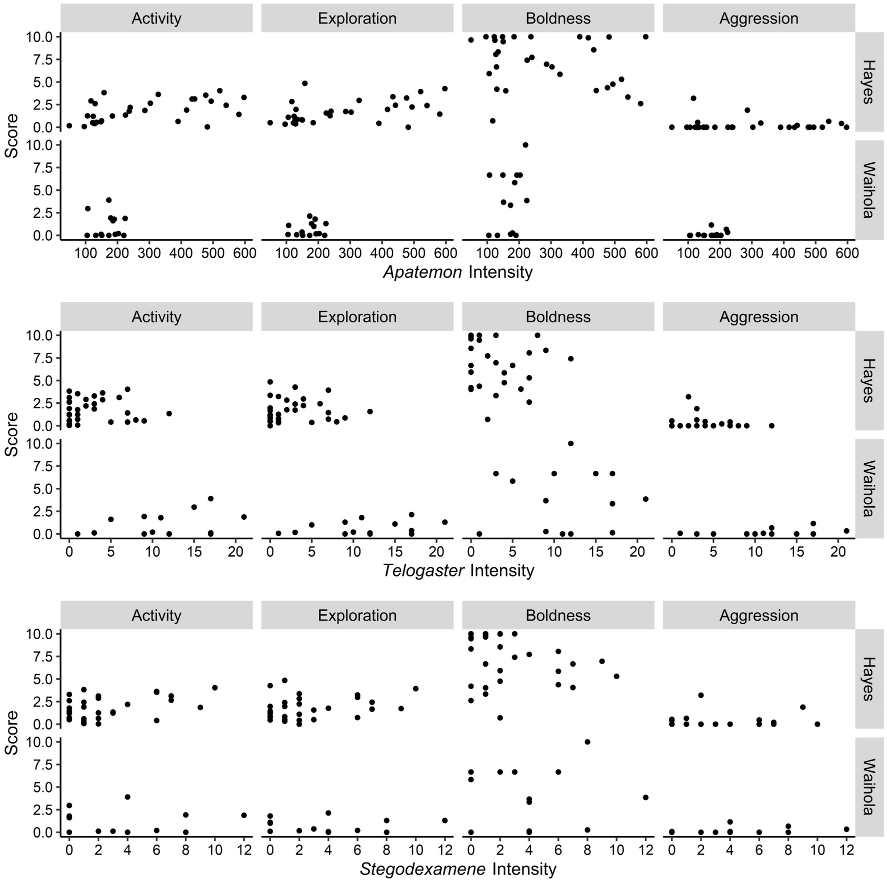

Figure 2. Scatterplots showing relationship between standardized scores for all behavioural traits of common bullies, *Gobiomorphus cotidianus*, and *Apatemon*, *Telogaster* and *Stegodexamene* intensities between Lake Hayes ($N = 31$) and Lake Waihola ($N = 14$) populations. Relationships with *Apatemon* intensity were significant ($p < 0.05$) for activity and exploration in Lake Hayes fish.

Overall, personality traits were repeatable between individuals, i.e., the 95% confidence interval of the repeatability estimate did not overlap zero. However, when each lake population is considered separately, boldness was not repeatable in Hayes whereas only activity was repeatable in Waihola (Figure 3). The individual consistency for boldness was predicted by *T. darbyi* intensity ($t = 2.637$, df = 24, $p = 0.014$) while individual consistency in activity was predicted by *S. anguillae* ($t = -2.226$, df = 24, $p = 0.036$) in the Hayes population (Figure 4; Table 3). Consistency of aggression in

Table 3.
Significant interactions ($p < 0.05$) of Between Population and within Hayes Population individual consistency models.

	Predictor	Estimate	S.E.	t	p
Between populations (df = 34)					
Boldness	(Intercept)	0.500	0.165	3.024	0.005
	Females: Waihola	0.760	0.368	2.065	0.047
Exploration	(Intercept)	0.644	0.168	3.826	0.001
Activity	(Intercept)	0.508	0.151	3.371	0.002
	Length: Hayes	0.031	0.015	2.127	0.041
Aggression	*Stegodexamene*: Hayes	0.130	0.054	2.408	0.022
Hayes (df = 24)					
Boldness	(Intercept)	0.487	0.105	4.625	0.000
	Tylodelphys	0.011	0.004	2.637	0.014
Exploration	(Intercept)	0.625	0.132	4.746	0.000
Activity	(Intercept)	0.544	0.104	5.216	0.000
	Stegodexamene	−0.063	0.028	−2.226	0.036
	Length	0.033	0.014	2.457	0.022
Aggression	(Intercept)	0.291	0.167	1.745	0.094
	Tylodelphys	0.018	0.007	2.690	0.013
	Stegodexamene	0.125	0.045	2.751	0.011

Waihola model included in Appendix B at 10.6084/m9.figshare.12860627.

the Hayes fish was also significantly predicted by *T. darbyi* ($t = 2.690$, df = 24, $p = 0.013$) and *S. anguillae* ($t = 2.751$, df = 24, $p = 0.011$) intensities (Figure 4; Table 3). All other parasite taxa had no impact on the within individual consistency of any personality trait (Figure 4; Appendix B at 10.6084/m9.figshare.12860627). However, larger fish in Hayes were more consistently active and boldness in Waihola was more repeatable in males than females (Figure 5; Table 3).

4. Discussion

Disease agents can influence animal behaviour by inducing alterations to individual personality traits (Bell & Sih, 2007; Dingemanse et al., 2007; Mikheev et al., 2010; Hammond-Tooke et al., 2012; Kortet et al., 2015). Those that invade sensory organs (e.g., eyes, brain, etc.) have the potential to be particularly impactful due to interfering with these processes (Barber & Crompton, 1997a, b; Barber & Wright, 2006). We have shown that an eye-invading parasite, *Tylodelphys darbyi*, is associated with boldness, activity,

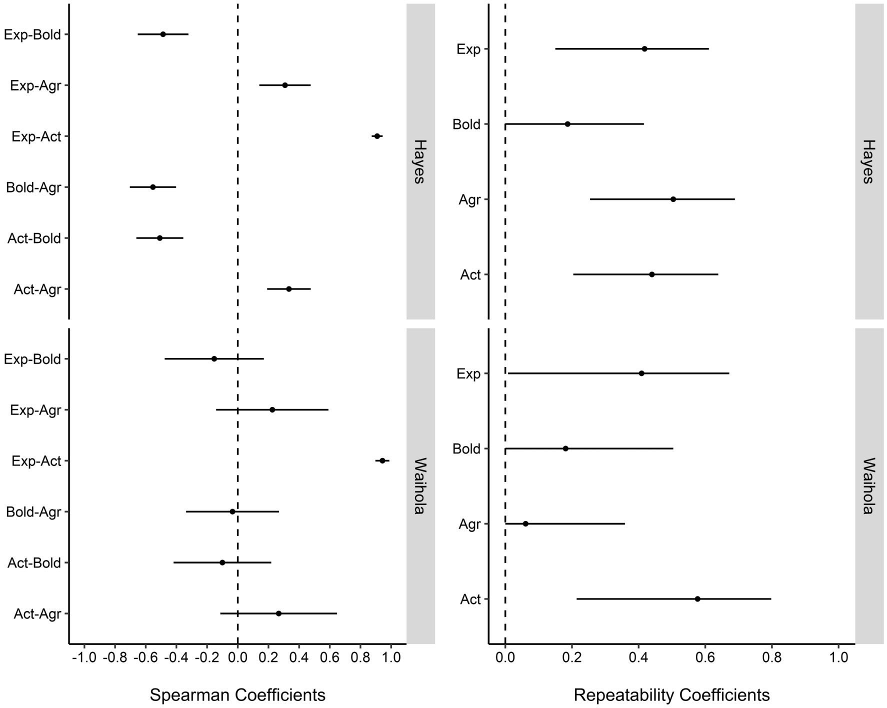

Figure 3. Spearman correlation coefficients between behaviours of common bullies, *Gobiomorphus cotidianus* (left), and repeatability estimates for each trait (right) with 95% confidence intervals for fish from Lake Hayes ($N = 31$) and Lake Waihola ($N = 14$).

and exploration in the fish *Gobiomorphus cotidianus*. As infection intensity with *T. darbyi* increases, the fish emerged from the shelter sooner after a predatory threat, as well as being more active and explorative. Only experimental infections could confirm the direction of causality. However, it seems more plausible and parsimonious to conclude that infection by a potentially vision-impairing parasite causes changes in behaviour, rather than particular behaviours lead to higher infection risk, especially given the reduced response to a simulated predator.

In addition, we see that as *Apatemon* sp. intensity increases so does activity level and exploration in the Hayes population. There were no significant relationships between personality traits and any parasites in Waihola even though *Apatemon* sp. is found in both lakes. However, we do see that behavioural correlations are different between lakes, with only exploration and activity positively correlating in Waihola. Likewise, only activity was repeat-

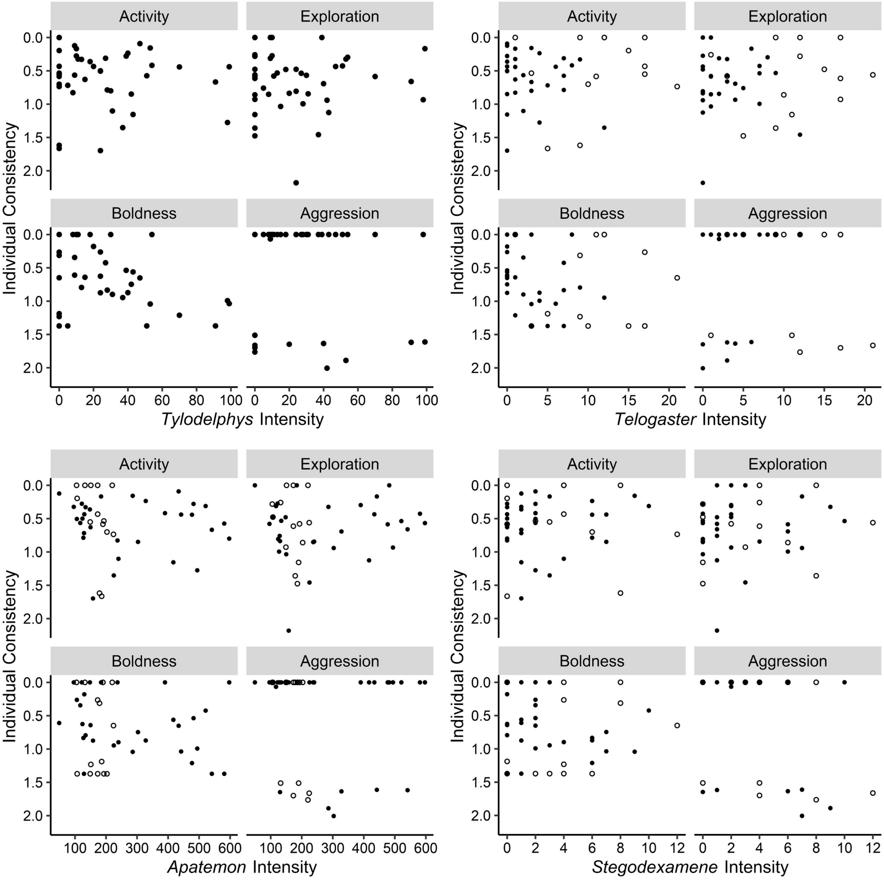

Figure 4. Scatterplots showing relationship between the individual consistency of all behavioural traits of common bullies, *Gobiomorphus cotidianus*, and *Tylodelphys*, *Apatemon*, *Telogaster* and *Stegodexamene* intensities for Lake Hayes ($N = 31$; ●) and Lake Waihola ($N = 14$; ○) populations. There are significant relationships between boldness consistency and *Tylodelphys* intensity as well as *Stegodexamene* intensity and activity consistency in the Lake Hayes fish. The y-axes are inverted to illustrate that larger values indicate a decrease in consistency.

able between individuals across sessions in Waihola fish, while boldness was the only trait not individually consistent over time in Hayes fish. In addition, Hayes fish with the most *T. darbyi* in their eyes are had lower consistency of boldness and those with high *S. anguillae* infections had slightly more consistent activity scores. Further, both *T. darbyi* and *S. anguillae* affected with the consistency of aggression in Hayes, but other than this behaviour being

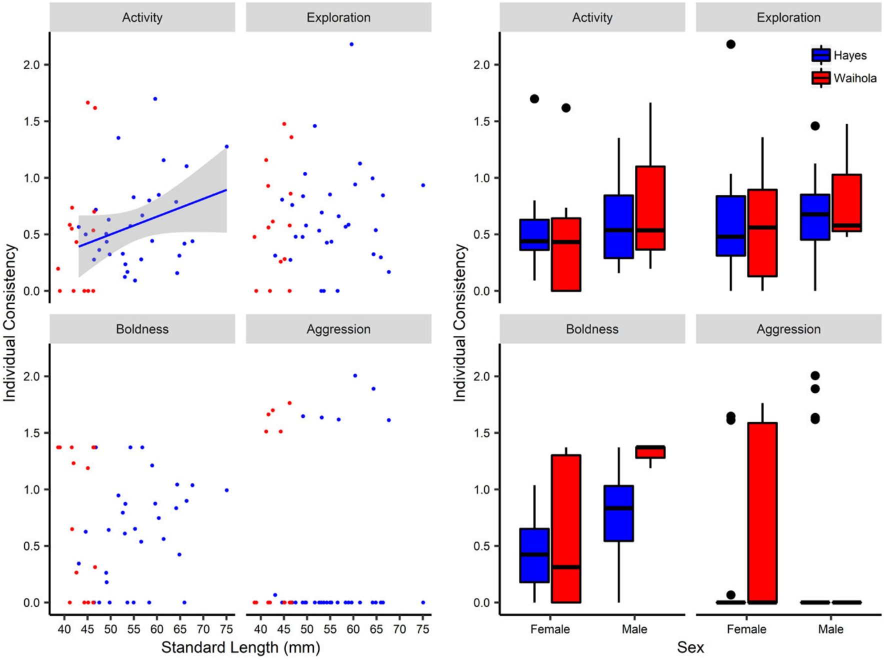

Figure 5. Within individual consistency of all behavioural traits of common bullies, *Gobiomorphus cotidianus*. Scatterplots on the left show the relationship between individual consistency and standard length for both Lake Hayes (blue) and Waihola (red) *G. cotidianus*. Boxplots on the right show differences in individual consistency between females and males as well as population.

inconsistent within individuals no pattern was clear (Figure 4). Our results are intriguing as previous studies on eye-infecting diplostomids have shown fish to be more aggressive, active, and bold, while other studies uncovered no association with activity, boldness or exploration (Mikheev et al., 2010; Klemme et al., 2016; Gopko et al., 2017). A previous study on *G. cotidianus* from Lake Waihola reported some associations between fish behaviour and infections by *Telogaster* and *Stegodexamene* (Hammond-Tooke et al., 2012), but our study found no relationships between parasitism and personality traits in the same population. Differential results within and among host as well as parasite species highlight the importance of undertaking multiple investigations with a variety of methods across new and familiar host-parasite systems.

In the Hayes population, we observed a potential association of *T. darbyi* and *Apatemon* sp. infection with multiple personality traits. Fish with higher intensities of *T. darbyi* emerged from shelter sooner, were more active, and more explorative, while *Apatemon* only influenced the latter two behaviours. Both species are trophically transmitted to the final host (i.e., bird) in their life cycles, therefore it is possible that these behavioural alterations aid in the parasite completing this step (Poulin, 2013; Presswell & Blasco-Costa, 2020). A previous study showed that bullies with the most *T. darbyi* tended to spend more time away from shelter moving about in the environment, corroborating our current findings, but interestingly there was no impact of *Apatemon* infection in that earlier study (Ruehle & Poulin, 2020). In this case, however, we have shown that both parasites could have multidimensional impacts on the behaviour of *G. cotidianus*, which could in turn have consequences for predator avoidance (Cézilly & Perrot-Minnot, 2005; Thomas et al., 2010; Cézilly et al., 2013). Bullies are benthic, using underwater structures for shelter, thus increased activity, exploration, and boldness might cause the fish to spend more time away from safety (Rahel & Stein, 1988; Ruehle & Poulin, 2020). Spending more time in the open and exiting a shelter soon after an attack could increase their vulnerability to predation due to being more conspicuous compared to hidden conspecifics (Lafferty & Morris, 1996; Fredensborg & Longoria, 2012). Ruehle & Poulin (2019) demonstrated that *T. darbyi* had no impact on the ability of infected bullies to avoid or react to a simulated predation event, so if there is any influence of parasitism it may involve a suite of subtle behaviours. Parasites may also alter the repeatability, or consistency, of personality traits rather than their magnitude, which could also act as a mechanism to facilitate trophic transmission (Coats et al., 2010; Poulin, 2013; Barber et al., 2017). In fish from Lake Hayes, the within individual consistency of boldness decreased with increasing *T. darbyi* intensity while activity was slightly more consistent with higher *S. anguillae* infections. A decrease in the consistency of boldness, or the time to emerge following an attack, while being consistently more active could facilitate bullies being eaten by the parasites' definitive hosts. However, an actual increase in predation to facilitate trophic transmission was not explicitly tested here and should be investigated further.

It is possible that the impacts on behaviour are a side-effect of infection rather than direct influence of the parasite, or even a result of innate host behaviour (Poulin et al., 1991; Wilson et al., 1993; Poulin, 2010). It is well known that parasites can inflict energetic demands on their hosts, resulting in a need to more actively forage (Toft, 1991; Barber et al., 1995, 2008; Sorenson & Minchella, 1998; Wood et al., 2007). Barber et al. (1995) demonstrated that infected sticklebacks spend more time foraging away from the protection of a shoal than uninfected fish. Bullies with the most parasites could simply be compensating for greater energetic demands by actively exploring the environment more than other fish. Likewise, by reversing the chain of causality, it is possible that more active fish encounter a larger number of infective stages than their conspecifics, with behaviour being the cause of infection rather than a direct result of infection (Poulin et al., 1991; Wilson et al., 1993). The infective stages of trematodes, the cercariae, are released from the first intermediate host, usually a snail, into the water column where they then, depending on the species, actively move about in search of the next host (Selbach & Poulin, 2018). Experimental infections would be needed to tease apart the differences between parasite-induced changes and innate behaviours of the host causing infection; however, despite intensive efforts to find the snail host of *T. darbyi*, it remains unknown.

Differences in personality traits between populations are well documented for several fish species (Bell & Sih, 2007; Dingemanse et al., 2007; Kortet et al., 2015). Here we found that the only behavioural correlation shared between *G. cotidianus* populations was the exploration-activity axis. This was the only correlation between behaviours in the Waihola population whereas all possible behavioural combinations were observed present in Hayes fish. Hammond-Tooke et al. (2012) found overall correlations between aggression-boldness and activity-aggression in Waihola fish, therefore the lack of relationships in the present study is interesting. Likewise, the only trait that was not repeatable across sessions in the Hayes fish was boldness whereas only activity was consistent in Waihola fish, again contrary to a previous study (Hammond-Tooke et al., 2012). These combined findings suggest not only different behavioural syndromes among populations, but also over time in the same population. Some behavioural differences between populations have been attributed to differential predation and/or infection risks (Kortet et al., 2010). We know that *T. darbyi* is not present in Lake Waihola not only because it has never been seen there despite years of study on that fish population, but also due to the absence of its definitive host, the crested grebe *Podiceps cristatus australis*. However, it is difficult to assess differential predation threat between populations as many aquatic predators (e.g., trout, cormorants, etc.) are shared between lakes (Jensen & Snoyink, 2005; Presswell & Blasco-Costa, 2019). Further, near-shore habitat variation (e.g., water depth, turbidity, amount of cover, etc.) could account for the population level differences seen here. Future studies would need to survey potential predators as well has habitat heterogeneity at both sites as well as parasite infection to further elucidate the potential drivers.

It is possible that the differences seen between populations are due to variation in the fish tested themselves. The lack of relationship among personality traits in Waihola in the present study may have been due to the small number of fish tested from this population compared to the previous study or to the sample size from Hayes. Further, sex ratios were different between the two lakes (i.e., Hayes = 42% female/58% male vs Waihola = 79% female/21% male) which could result in variation between personality traits given there is evidence of sex specific behaviour during the breeding season (e.g., male aggression toward conspecifics; McDowall, 1990). Another explanation could be the differing collection methods between the lakes, as we used traps in Hayes and a seine net in Waihola. It has been shown that trapped fish tend to be bolder than those that do not enter traps (Wilson et al., 1993). Unfortunately, seine netting in Hayes is difficult to impossible around most of the lake margins due to depth, substrate (i.e., large rocks), and the local bullies not being aggregated, therefore the use of traps was necessary. Likewise, Lake Waihola is frequented by visitors and has a sufficient eel population that traps face the risk of tampering or captured fish being eaten before collection, making seining the viable option.

Multidimensional assessments of parasitic influences on host behaviour are becoming more common (Coats et al., 2010; Hammond-Tooke et al., 2012; Kortet et al., 2015; Klemme et al., 2016; Gopko et al., 2017). Such undertakings are important as host-parasite interactions are rarely so simplistic as to result in changes to a single host behavioural axis. We have demonstrated that several parasite taxa, including the eye-dwelling *T. darbyi*, have the potential to influence up to 4 different personality traits related to personality, in a locally ubiquitous fish species as well as some possible population level differences hinting as divergent behavioural syndromes. In order to further investigate this system, experimental infections will be an imperative once the snail host of *T. darbyi* has been identified. Nevertheless, using wild fish with natural infection levels is meaningful for establishing how these organisms interact naturally and identify infection-behaviour associations. In doing so, we have revealed population-dependent patterns highlighting how parasitism may differentially interact with key components of natural ecosystems.

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