Received: 22 March 2019 | Revised: 27 June 2019 | Accepted: 28 June 2019
DOI: 10.1111/eth.12927

RESEARCH PAPER

ethology WILEY

No impact of a presumed manipulative parasite on the responses and susceptibility of fish to simulated predation

Brandon Ruehle | **Robert Poulin**

Department of Zoology, University of Otago, Dunedin, New Zealand

Correspondence
Brandon Ruehle, Department of Zoology, University of Otago, Dunedin, New Zealand.
Email: brandon.ruehle90@gmail.com

Funding information
University of Otago

Editor: T. Tregenza

Abstract

Parasites manipulating their host to facilitate trophic transmission is a widespread and diverse phenomenon. Trematode eye-flukes in the family Diplostomidae infect a variety of fish species as metacercariae, many residing in the eyes. A recently described diplostomid, *Tylodelphys darbyi*, from the South Island of New Zealand has been found to infect common bully *Gobiomorphus cotidianus*, an endemic freshwater fish. Within the fish, the metacercariae move about freely in the liquid parts of the eye and are quite large. We hypothesized that increasing intensity of *T. darbyi* infection will result in increasing visual impairment, thus reducing the ability of *G. cotidianus* to identify and react to a predatory threat. To test this hypothesis, we performed experiments to (a) examine the fish's reaction to a purely visual predator cue and (b) test their ability to avoid simulated predation under natural levels of infection. Among the 64 fish used in our experiments, *T. darbyi* had a prevalence of 98.7% with an average of 17.6 worms per fish. However, there was no relationship between *T. darbyi* intensity and either the fish's reaction to a visual predator stimulus or their ability to escape a simulated predator. Our findings indicate that despite being present in large numbers in the eyes of its fish host, the parasite appears incapable of improving its chances of trophic transmission to its avian definitive host. The results also suggest that the fish *G. cotidianus* could be using other senses (e.g., olfaction and lateral line) to compensate for visual impairment, and detect and respond to predators.

KEYWORDS
diplostomid, eye-fluke, *Gobiomorphus cotidianus*, host manipulation, host–parasite interactions, trophic transmission

1 | INTRODUCTION

The manipulation of a host organism by its parasites is a widespread phenomenon that varies in its expression across taxa and interactions, from helminths that cause corals to swell and turn pink to fungi that move their hosts to vantage points ideal for spore dispersal (Aeby, 2002; Andersen et al., 2009; Holmes & Bethel, 1972; Moore, 2002, 2013; Poulin, 2010). However, classifying a behavioural change as a parasite "manipulation" has not been the easiest of tasks. Poulin (2010) defines manipulation as any interaction that alters the host phenotype (behavioural, physical or otherwise) in ways that increases the fitness of the parasite. Within this definition, four general types of manipulation were identified, and of these, the most dramatic occurs when a parasite requires its current host to be ingested by another to complete its life cycle (Poulin, 2010). Termed parasite-increased trophic transmission, this form of manipulation has been a hot topic in recent years (Lafferty, 1999; Moore, 2002, 2013; Poulin, 2010).

In trematodes, manipulation often occurs in one of the intermediate hosts (Aeby, 2002; Carney, 1969; Poulin, 2010; Wesolowska &

Wesolowski, 2014). A generalized three-host life cycle starts with a miracidium entering a snail, 1st intermediate host, developing into a colony of sporocysts, and asexually producing cercariae (Galaktionov & Dobrovolskij, 2003). Cercariae are then released into the environment to seek out the 2nd intermediate host in or on which they encyst as metacercariae to await ingestion by the definitive host where the parasite matures and reproduces (Galaktionov & Dobrovolskij, 2003). In many cases, it is this step from 2nd intermediate host to definitive host that is considered an unlikely event without the parasite changing the odds in its favour (Moore, 2002; Poulin, 2010). A variety of trematode parasites utilize fish as 2nd intermediate host, and as such, they are prime targets for manipulation (Barber, 2007; Barber, Hoare, & Krause, 2000; Barber & Wright, 2006). A classic example of manipulation in fishes is seen in North American killifish *Fundulus* spp. infected with *Euhaplorchis* trematodes (Fredensborg & Longoria, 2012; Lafferty & Morris, 1996). Metacercariae of *Euhaplorchis* encyst in the brains of killifish which serve as the 2nd intermediate host for the parasite (Lafferty & Morris, 1996; Martin, 1950). Infected individuals showed conspicuous behaviours (e.g., contorting, surfacing and flashing) that increased in frequency with higher intensities of infection and, as a result, were captured at much higher rates by birds, the definitive host, than uninfected fish (Fredensborg & Longoria, 2012; Lafferty & Morris, 1996). Encysting in the brain is not uncommon for trematodes, and the damage caused can affect how the host moves, as in the killifish, or alter the efficacy of sensory organs (Barber & Crompton, 1997a, 1997b). However, some trematode taxa forego this indirect approach and invade the sensory organs themselves.

Trematodes in the family Diplostomidae infect a variety of fish species as metacercariae and can be found, among other areas, in the eyes (Karvonen, Paukku, & Seppälä, 2005; Seppälä, Karvonen, & Valtonen, 2004, 2005, 2008; Stumbo & Poulin, 2016). Some diplostomids, such as *Diplostomum* spp., are found in the lens of the fish eye, wherein metacercariae release waste products that result in cataracts (Karvonen, Seppälä, & Valtonen, 2004; Shariff, Richards, & Sommerville, 1980). These cataracts have been shown to negatively impact predator avoidance behaviours of rainbow trout *Oncorhynchus mykiss*, making infected fish easier prey (Seppälä, Karvonen, & Valtonen, 2004, 2005, 2008). Closely related taxa in the genus *Tylodelphys* also inhabit the eyes of fishes but are more often found in the humours (i.e., the fluid) when present in the eye, and since this is more spacious compared to the lens, the parasites are able to move about freely (Blasco-Costa, Poulin, & Presswell, 2017; Burrough, 1978; Chibwana, Nkwengulila, Locke, McLaughlin, & Marcogliese, 2015; Drago & Lunaschi, 2008; Flores & Semenas, 2002; Garcia-Valera, Sereno-Uribe, Pinacho-Pinacho, Hernandez-Cruz, & Perez-Ponce de Leon, 2016; Kennedy, 1987). Unlike *Diplostomum* spp., however, *Tylodelphys* is relatively understudied for its ability to impact fish behaviour (Munoz, Staaks, & Knopf, 2017 is a notable exception), in particular the effect these parasites might have on their hosts' ability to avoid predators.

Recently, a new species of *Tylodelphys* was described in New Zealand from lakes on the South Island (Blasco-Costa et al., 2017). As with many diplostomid trematodes, *Tylodelphys darbyi* has a three-host life cycle, first utilizing an unknown snail, likely a lymnaeid, then the common bully *Gobiomorphus cotidianus* and finally the Australasian great crested grebe *Podiceps cristatus australis* as the definitive host (Blasco-Costa et al., 2017; Presswell & Blasco-Costa, 2019; Stumbo & Poulin, 2016). Within the fish, *G. cotidianus*, *T. darbyi* metacercariae inhabit the vitreous and aqueous humours of the eye, moving freely between the two; they can reach lengths greater than 1mm and may occur in high intensities, for example often >10 metacercariae per eye (Blasco-Costa et al., 2017; Presswell & Blasco-Costa, 2019). Recently, Stumbo and Poulin (2016) demonstrated that *T. darbyi* shows a diel behaviour pattern in which individuals moved farther up in the eye during the day, potentially increasing obstruction, and retreated to the bottom of the eye at night. The authors hypothesized that this diel movement could increase the likelihood of transmission during the day when grebes are active by impairing the vision, and consequently the evasiveness, of the bullies (Stumbo & Poulin, 2016).

Gobiomorphus cotidianus is the most widespread bully species in New Zealand, occurring across freshwater streams, lakes and estuaries (McDowall, 1990; Michel et al., 2008; Vanderpham, Nakagawa, & Closs, 2013). At present, only *G. cotidianus* from a single lake, Lake Hayes, in the Otago region of the South Island have been identified as infected with *T. darbyi* (Blasco-Costa et al., 2017; Presswell & Blasco-Costa, 2019; Stumbo & Poulin, 2016). As for the only identified definitive host, in New Zealand, *P. cristatus australis* is only found on lakes of the South Island and, like many native birds, is considered nationally vulnerable (Presswell & Blasco-Costa, 2019; New Zealand's Department of Conservation). Considering the ubiquity of the 2nd intermediate host and limited range of the definitive host, understanding how, if at all, *T. darbyi* impacts the behaviour of *G. cotidianus* could have intriguing implications for aquatic ecology in New Zealand. In the present study, we hypothesize that increasing intensity of *T. darbyi* infection will result in increasing visual impairment, thus reducing the ability of *G. cotidianus* to identify and react to a predatory threat. To test this hypothesis, we performed experiments to (a) examine the fish's reaction to a purely visual predator cue and (b) test their ability to avoid simulated predation under natural levels ofinfection.

2 | METHODS

2.1 | Collection, housing and tagging

Common bullies *Gobiomorphus cotidianus* (n = 64; Animal Ethics Committee approval # 70/17001) were collected from Lake Hayes, Otago on the South Island of New Zealand in the Summer and Spring of 2018. Hayes is a small lake (~2.76 km^2; Otago Regional Council) located in the Southern Alps approximately 272 km from the University of Otago. Minnow traps were deployed over night and collected the following morning. Fish were transported in ice chests provided with air stones back to the university and immediately transferred to holding tanks (see dimensions below). All holding tanks were filled with 1/3 saltwater and 2/3 freshwater (isotonic solution) to prevent fungal growth on bullies and provided with filtration and aeration. The bullies were fed ad libitum daily on commercial fish pellets, and excess food was removed via suction tube. Fish were allowed 72 hr to acclimate to holding conditions (20°C room temperature water; 12-hr photoperiod) before receiving, under anaesthesia (5 min in a MS-222 solution, 1 mg/L), a unique 2mm visible implant elastomer (VIE) tag for identification purposes (Northwest Marine Technologies Inc.). These implants were inserted at least 2 weeks before any behavioural experiment.

2.2 | Predator stimulus

Two aquaria (14 L; 31.5 cm × 19 cm × 14.5 cm) were utilized as testing arenas, both covered on three sides with black plastic to reduce outside stimuli during trials. For this experiment, a simulated predator in the form of a shadow passing through the tank was generated by a computer and presented to the fish using a mobile tablet (Galaxy S). First, the tablet was wirelessly connected to a laptop PC (Dell Intel Core i5-72000U) using the Spacedesk ×10 app (datronicsoft Inc.) to be used as an extended monitor and then placed over the top of the arena. The PowerPoint shown on the tablet was split into black and white halves to create light and dark sides of the arena; the white half being where the rectangular shadow passed through. By splitting the tank in half, we were able to simulate open space (i.e., the lit area) and cover (e.g., large rock and sunken log) using the dark side. To decrease light penetration into the dark half of the tank, and further simulate cover, a vertical black divider was placed at the boundary between halves, stopping 8 cm above the tank bottom to allow the fish to move from one half to the other. As only one tablet was available, it was moved from arena to arena as needed.

Fish from the Summer collection (*n* = 33) were used for this experiment and were held in groups of 3 in separate 14 L aquaria (31.5 cm × 19 cm × 14.5 cm). Each fish was used twice with a minimum of 48 hr in between trials and testing occurred over a 2-week period. A coin was flipped twice for each trial, once to determine which arena was used and again to decide the side of the tank to be lit. Following this, the test fish was introduced to the arena in a glass jar, that was then gently inverted and lifted to release the fish, and a glass cover supporting the tablet was set directly over the opening. All lights in the room were off and a black plastic sheet separated the test arenas from the observer operating the laptop. A 15-min acclimation period was followed by 15 min of filming, 10 min pre-stimulus and 5 min post-stimulus (Sony Handycam FDR-AX33). The shadow was timed to pass through the tank at the 10-min mark during the trial. In total, the fish were in the arena for 30 min and promptly placed back into their holding tanks afterwards. From the videos, a fish's reaction was scored (0 = no reaction, 1 = paused, 2 = immediate use of cover) and the duration (s) of the pause if it occurred, was recorded. A "pause" was defined as the fish ceasing movement and sitting motionless on the bottom. During data analysis, the two types of reactions were pooled such that the response of the fish was treated as a binomial (0 = no reaction, 1 = reaction). A further analysis comparing the two reaction types (i.e., pause vs. escape to cover) was not included due to the small number of "escape" responses (*n* = 4).

2.3 | Predator avoidance

Bullies (originally *n* = 34, for data analysis *n* = 31) for this experiment were collected in Spring 2018 and held in sized matched groups (*n* = 6 individuals per group) in separate holding tanks (36 L; 44 cm × 27.5 cm × 29.5 cm). Each group started with six fish except for one which had 5, due to collection numbers. One fish died before the completion of the experiment, so its group was also treated as having five individuals. Further, mortality after the experiment, but before dissection for parasites, resulted in the final number of fish (*n* = 31) used for data analysis. Two large (54 cm × 80 cm × 28 cm) plastic tanks were used as testing arenas, each provided with three pieces of 10-cm-long PVC pipe for cover. Each group of fish was used in six trials, with the first set of three trials taking place at 2-day intervals, and the second set of three trials starting after a 7-day rest period and also taking place at 2-day intervals. In any given trial, a single group was placed in a given arena 48 hr before the trial began to acclimate and then allowed an additional 48 hr between each trial in a set. Each trial consisted of a volunteer using a net (7.5 cm × 10 cm) to capture fish in a simulated predation event; volunteers were chosen randomly, not allowed to participate more than twice in a row, and never used in more than three trials. The volunteers were allowed to freely pursue fish for 5-s intervals with 10s of rest (i.e., the net is out of the water) in between for a maximum of 20 attempts, until all fish were captured, or all fish were inside the cover. Bullies were scored following each trial from 6 to 0 corresponding to the order of capture (e.g., 6 = first, 1 = last and 0 = not caught; with the exception of groups with 5 in which 5 = first); the number of times each fish was captured over the course of the 6 trials was also recorded. The score for each fish was standardized using the following formula to produce a single value for each individual and account for variable group size (5–6 fish): $\frac{10\,(\text{Mean score over 6 observations})}{\text{Number of fish in the group during the observations}}$. This formula generates values from 0 to 10 such that a fish caught first every time would have a score of 10, regardless of the number of fish in that group, and those never caught would have a 0. Individuals that died prior to dissection for parasite counts were excluded from data analysis but the scores assigned to other fish within their group during the experiment remained the same (i.e., if the group had 6 fish during testing but one died "6" would still represent first capture for analysis).

2.4 | Parasite counts

Following the experiments, all fish were euthanized by overdose of MS-222 (10 mg/L), measured for standard length (SL) and weighed (TM), then immediately decapitated. The eyes were removed and examined separately to obtain numbers of *Tylodelphys darbyi* for both

TABLE 1 Prevalence (%), mean intensity and the range of intensity for all parasite taxa from both collections as well as overall

				Intensity range	
Collection	**Helminth taxa**	**Prevalence (%)**	**Mean intensity**	**Min**	**Max**
Summer (*n* = 33)	*Tylodelphys darbyi*	97.0	8.4	0	27
	Apatemon sp.	100.0	220.1	72	516
	Telogaster opisthorchis	39.4	3.8	0	11
	Stegodexamene anguillae	12.1	1.0	0	1
	Eustrongylides sp.	3.0	1.0	0	1
Spring (*n* = 31)	*Tylodelphys darbyi*	100.0	29.8	5	67
	Apatemon sp.	100.0	236.4	62	469
	Telogaster opisthorchis	83.9	7.2	0	70
	Stegodexamene anguillae	87.1	5.7	0	25
	Eustrongylides sp.	32.3	1.3	0	2
Overall (*n* = 64)	*Tylodelphys darbyi*	98.7	17.6	0	67
	Apatemon sp.	100.0	236.2	62	516
	Telogaster opisthorchis	57.9	5.8	0	70
	Stegodexamene anguillae	43.4	5.0	0	25
	Eustrongylides sp.	15.8	1.3	0	2

the left and right eyes. We focused on the humours in the eyes, as this is where *T. darbyi* is found, but the lens and other tissues were also examined. After dissecting the eyes, the rest of the fish was examined for total parasite burden. Several trematodes, *Apatemon* sp., *Telogaster opisthorchis* and *Stegodexamene anguillae*, are commonly found encysted as metacercariae in muscle tissues (e.g., body and head), connective tissues in the body cavity and various organs (e.g., gonads and liver). The nematode *Eustrongylides* sp. is also known to be found in the body cavity of *G. cotidianus* so their presence was recorded as well. For all parasite taxa, in addition to the infection intensity of individual fish, we also calculated prevalence (proportion of individual fish infected) and mean intensity of infection (mean number of parasite individuals per infected fish) (Bush, Lafferty, Motz, & Shostak, 1997).

2.5 | Statistical analysis

Statistical tests were performed, and figures generated, using the *lme4*, *lmerTest* and *ggplot2* packages in the program R (version 3.5.2; R Core Development Team, 2018). All models include the number of *T. darbyi* (i.e., the intensity of infection per individual fish) as main predictor, as it is the taxon under scrutiny. We also tested for the effect of *Apatemon* sp.; however, other parasites were excluded as they were rare or too few by comparison (see results; Table 1). We used a generalized linear mixed-effects model (GLMM; *glmer* function) and a linear mixed-effects model (LMM; *lmer* function) both with fish ID as a random factor, to account for using the same individual more than once, and intensities of *T. darbyi*, *Apatemon* sp. and fish SL as predictors for the predator stimulus experiment. The GLMM, run with the binomial distribution, had the fish's reaction (i.e., no reaction vs. reaction) as the response variable, and for the LMM, we used the duration of the pause as the response variable. Further, LMMs were used for the predator avoidance experiment, using group ID as a random factor. For these models, standardized score and number of captures were used as response variables with *T. darbyi* intensity, *Apatemon* intensity and SL as predictors. Beyond the models addressing the main experiments, we also ran Pearson correlations to test for a relationship between left and right eye *T. darbyi* intensities (i.e., to determine whether infection was evenly distributed between the eyes), for a relationship between SL and either *T. darbyi* or *Apatemon* intensities, and for a relationship between the number of captures and standardized scores, using the *cor.test* function.

2.6 | Ethical note

We used naturally infected fish for our experiments, because the complete life cycle of *Tylodelphys darbyi* is not known. For this reason, we aimed to minimize our sample sizes while keeping them large enough to achieve statistical requirements. *Gobiomorphus cotidianus* is the most abundant and widespread endemic, freshwater fish in New Zealand (McDowall, 1990) and >100 can be recovered in a single trap night, so the numbers we used per experiment (<40) are not likely to impact the population. In addition, the Lake Hayes population has a *T. darbyi* prevalence of near 100% (Stumbo & Poulin, 2016; current study), allowing us to collect smaller numbers of fish and still guarantee infection. Fish were kept in holding tanks at stocking densities of <2 L/fish and provided with enough shelter (i.e., 10-cm PVC pipes) that each individual had a territory to itself. Common bullies take longer to acclimate (e.g., pale colouration) to holding conditions when not in a group and without adequate shelter (author per. observation), so these conditions are intended to reduce stress. For each experiment, fish were held in captivity for no more than 4 months at the end of each all individuals were humanely killed via overdose of MS-222 (10 mg/L). These methods were approved by the University of Otago Animal Ethics Committee (# 70/17001).

3 | RESULTS

We examined 64 *Gobiomorphus cotidianus* for parasite load over the course of these two experiments and recovered five taxa of parasitic helminths (Table 1). *Tylodelphys darbyi* was found in almost every individual, with an average of about 17 worms per fish (see Table 1). Most *T. darbyi* specimens were recovered from the humours of the eyes, but 13 individuals were found in the braincase while dissecting fish collected in Spring (p = 25.8%). For both collections, the intensity of *T. darbyi* showed a significant, positive correlation between left and right eyes (Pearson correlation: $r = .90$, $N = 64$, $p < .001$; Figure 1). The other trematode taxa recovered across both collections were *Apatemon* sp., *Telogaster opisthorchis* and *Stegodexamene anguillae*. *Apatemon* sp. was found in every fish sampled at very high intensities while *T. opisthorchis* and *S. anguillae* had lower prevalence and intensities (Table 1). All three of these taxa were found in the muscles and connective tissues, but *T. opisthorchis* and *S. anguillae* could also be found in various organs (e.g., liver and gonads). *Eustrongylides* sp. was the only nematode found in the fish examined and always occurred as larvae associated with tissues surrounding the alimentary canal. There was a significant, positive correlation between *T. darbyi* intensity and fish SL (Pearson correlation: $r = .54$, $N = 64$, $p < .001$; Figure 2), but not between *Apatemon* sp. intensity and SL (Pearson correlation: $r = .20$, $N = 64$, $p = .12$).

There was no significant effect of *T. darbyi* intensity on whether or not fish reacted to the simulated predator (binomial GLMM: $z_{57} = -1.28$, $p = .14$), with both reacting and non-reacting fish having similar *T. darbyi* intensities (Figure 3). Likewise, the duration of the pause, if it occurred, was not significantly predicted by *T. darbyi* intensity (LMM: $t_{5.26} = 0.45$, $p = .67$; Figure 4). The intensity of *Apatemon* sp. also did not predict a difference between hosts' reaction (binomial GLMM: $z_{57} = -1.14$, $p = .27$) and the duration of the pause (binomial GLMM: $t_{5.89} = -0.72$, $p = .50$).

For the predator avoidance experiment, we tested 31 *G. cotidianus*. The intensity of *T. darbyi* did not significantly predict the standardized score (LMM: $t_{25} = 0.42$, $p = .68$; Figure 5) or the number of times an individual was captured (LMM: $t_{25} = 0.82$, $p = .41$; Figure 5). Similarly, *Apatemon* sp. intensity did not predict an individual's score (LMM: $t_{25} = -0.78$, $p = .44$) or number of captures (LMM: $t_{25} = -0.99$, $df = 25$, $p = .33$). Standard length was not a significant predictor of either response variable (LMM score: $t_{25} = -0.65$, $p = .52$; LMM captures: $t_{25} = -1.40$, $p = .17$). However, there was a significant positive correlation between an individuals' score and the number of times it was caught (Pearson correlation: $r = .95$, $N = 31$, $p < .001$; Figures 6 and 7).

4 | DISCUSSION

Manipulation of the host by a parasite to facilitate trophic transmission has been heavily studied. It is suggested that those parasites residing in sensory organs (e.g., brain and eyes) can impact the behaviour of the host due to interference with their processes (Barber & Crompton, 1997a, 1997b; Barber & Wright, 2006). However, in this case we did not find a relationship between an eye-fluke, *Tylodelphys*

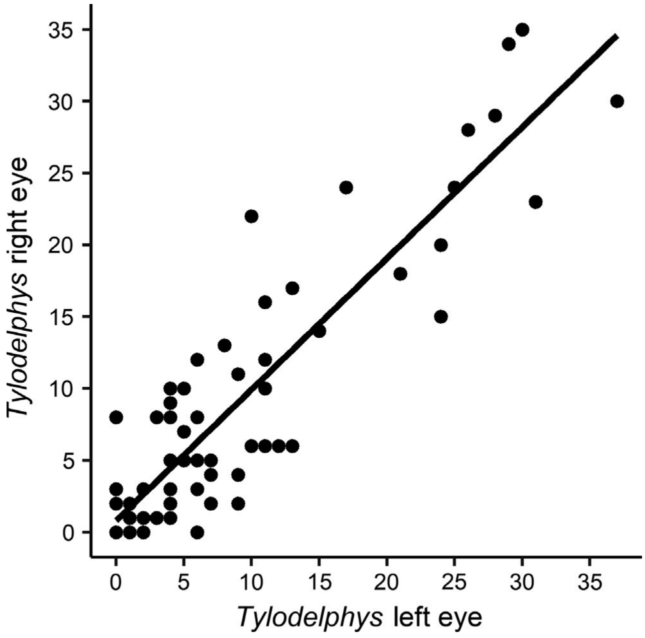

FIGURE 1 Mean intensity of *Tylodelphys darbyi* infection in right and left eyes

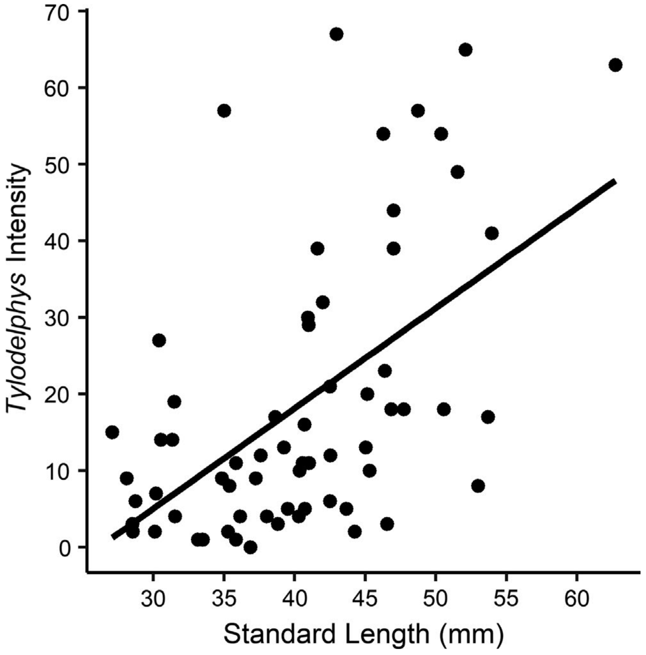

FIGURE 2 Scatterplot showing relationship between standard length and *Tylodelphys darbyi* intensity

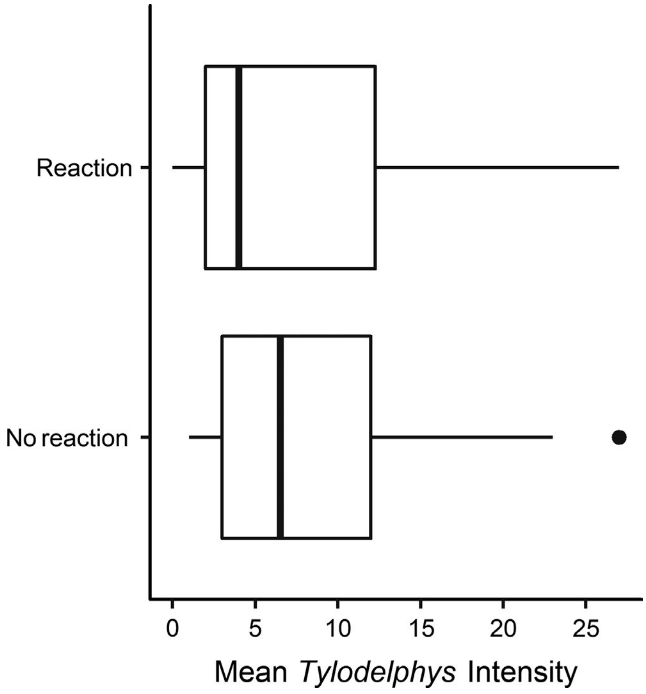

FIGURE 3 Mean intensity of *Tylodelphys darbyi* of those individuals that reacted to the stimulus and those that did not

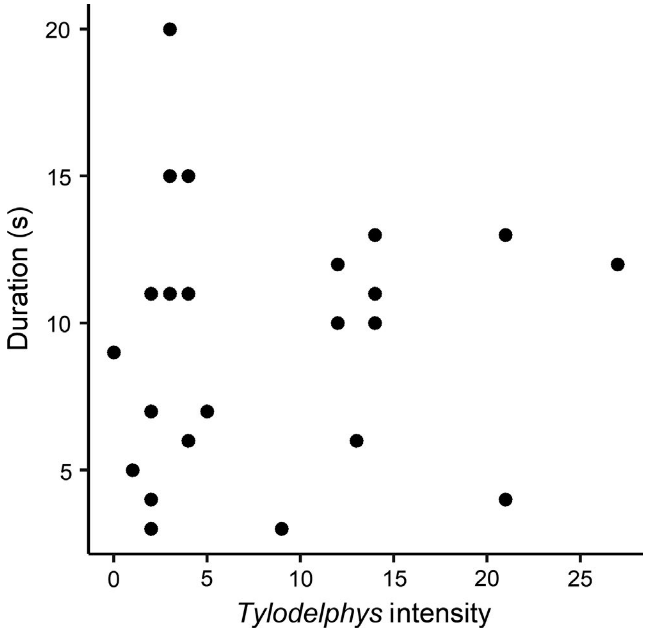

FIGURE 4 Scatterplot showing the relationship between the duration of the pause in reaction to the predator stimulus and *Tylodelphys darbyi* intensity

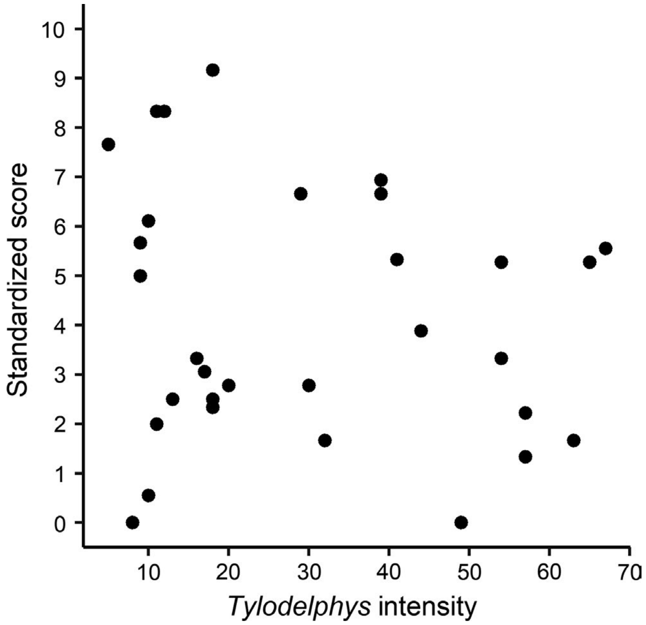

FIGURE 5 Scatterplots showing the relationship between the *Tylodelphys darbyi* intensity and the standardized score (left) and average number of captures (right) for each common bully

darbyi, and either the reaction to a visual predator stimulus or the ability of the host, *Gobiomorphus cotidianus*, to escape a simulated predator, even when infection intensity was high. We did find a positive correlation between the left and right eye intensities of *T. darbyi*, indicating that the parasite load is evenly split between both eyes, a result consistent with previous findings (Stumbo & Poulin, 2016). The positive correlation between the size of the fish and *T. darbyi* intensity is not surprising and is a common trend with larger individuals harbouring more parasites than smaller ones (Kuris, Blaustein, & Alio, 1980; Poulin, 2000; Poulin & George-Nascimento, 2007; Ruehle, Herrmann, & Higgins, 2017). In addition, we found no relationship between the intensity of the most common parasite found in the bullies, *Apatemon* sp., and our measures of predator avoidance, even though this parasite must also be transmitted by predation to a bird definitive host to complete its life cycle. For *T. darbyi*, these results are unexpected not only due to the intensities in which they occur in individual eyes, but also with respect to their size and mobility. Individual metacercariae can be seen moving within the eyes of live bullies (e.g., in front of and behind the lens) without magnification, even when looking at fish in the aquarium (Ruehle, B., observation). The lack of uninfected fish in our study means that we could only assess possible manipulation of the host via a relationship between host behaviour and intensity of infection, and not by comparing behaviour between infected and uninfected fish. Nevertheless, the former is almost as good a test for manipulation as the latter.

The highly mobile nature of *T. darbyi*, unlike, for example, *Diplostomum* spp. which often reside in the lens, could be an explanation for our results. When in the lens, *Diplostomum* induces cataracts as a result of waste products released by the parasite, and these can be quite extensive at high intensities of infection (Karvonen et al., 2004; Seppälä, Karvonen, & Valtonen, 2011). Fish lenses are transparent spheres that focus light passing into the eye before it reaches the retina, and cataracts reduce that transparency (Fernald, 1990). Therefore, it stands to reason that the associated pathology of *Diplostomum* would impact the ability of the host to visually react to a predatory stimulus (Seppälä, Karvonen, & Valtonen,

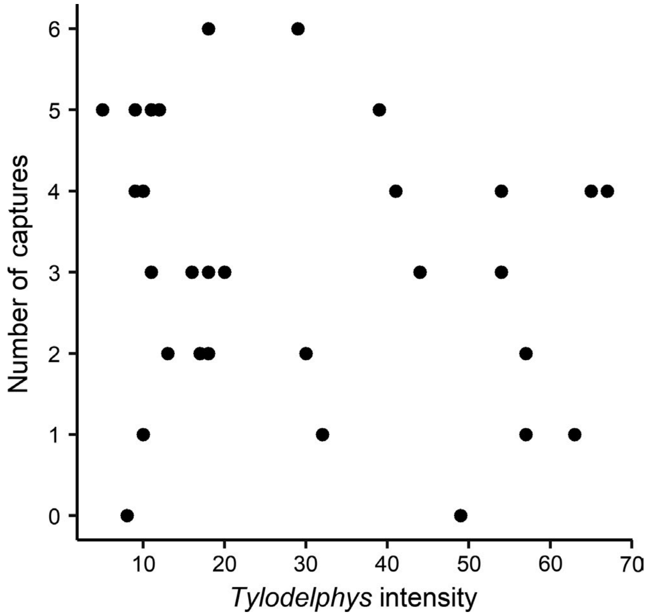

FIGURE 6 Relationship between *Tylodelphys darbyi* intensity and the average number of captures for each common bully

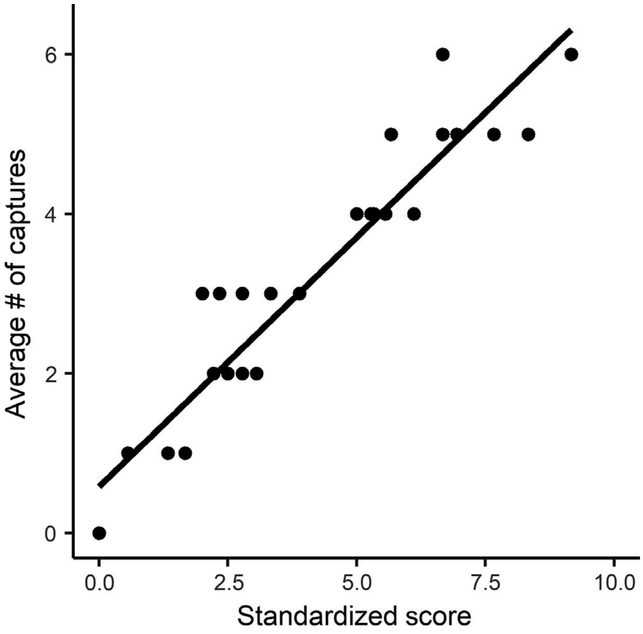

FIGURE 7 Relationship between the average number of captures and standardized score for each common bully

2004, 2005, 2008). By contrast, *T. darbyi* appears to have very little pathological impacts on the host (e.g., no damage to the retinal tissues; Stumbo & Poulin, 2016) other than being large, highly mobile (for a worm) and obstructing of the retina. Stumbo and Poulin (2016) showed that retinal obstruction is extensive during the day, when our experiments took place, so it is unlikely the diel movements of *T. darbyi* impacted our results. It could be that the near 100% prevalence of infection in this population masks impacts that would be seen in localities with more uninfected individuals. However, considering parasites are often aggregated within a host population (i.e., most parasites are found in few hosts) there should still be more bullies with lower levels of infection than those with high (Anderson & May, 1978), suggesting that the biology of *G. cotidianus* itself could explain our findings.

We utilized a purely visual predator simulation in the Predator Stimulus experiment, something *G. cotidianus* are unlikely to encounter in the wild. Aside from vision, fishes possess complex, and varied, chemo- (e.g., olfactory) and mechanosensory (e.g., the lateral line) systems for detecting stimuli, such as predatory threats (Barber & Wright, 2006; Bleckmann, 1986; Bleckmann & Zelick, 2009; Chivers & Smith, 1993; Hara, 1986). For example, the response of bullies to predators has been studied using water scented with shortfin eel *Anguilla australis* (i.e., a native predator; Hammond-Tooke, Nakagawa, & Poulin, 2012) and perch *Perca fluviatilis* (i.e., an introduced predator; Vanderpham, Nakagawa, & Clossy, 2012). Vanderpham et al. (2012) showed that *G. cotidianus* utilized shelter (i.e., PVC tubes) more in the dark when a predator odour was present compared to the control, but contrary to their predictions there was no difference between control and odour treatments in light conditions. The authors proposed that perhaps the lack of visual cues in addition to predator odour produced the unexpected results. Additionally, the lateral line of fishes detects pressure waves and other movements in the water column, such as those caused by other animals (Bleckmann, 1986; Bleckmann & Zelick, 2009). A real predator would generate a disturbance in the water that *G. cotidianus* could potentially detect, but, in this case, there was no movement in the tank other than the fish itself. Therefore, the lack of any other stimulus (e.g., predator odour and water disturbance) resulting in an unrealistic predatory event could be the reason for the apparent lack of response by the bullies rather than *T. darbyi* having no impact. Having said this, visual cues can serve a fish in shallow water to detect an avian predator even before the latter lands on the water surface, and the apparent unresponsiveness of bullies, even lightly infected ones, to visual stimulation is puzzling.

The second experiment aimed to simulate an actual predation event by capturing the bullies with a small aquarium net. In doing so, we solved the issue discussed above of only having a single predator cue since the fish not only could see the net but also detect its movement with their lateral line. We did not observe any relationship between *T. darbyi* intensity and either the score of the fish (based on order of capture, and therefore on relative susceptibility with respect to its group membership) or the number of times an individual was caught, but there was a significant positive correlation between the score and number of captures. This shows that the individuals that were captured most often were also caught earlier in the trials; however, the reason for this remains unclear. It is possible that the lack of a relationship between *T. darbyi* and the risk of being caught by a predator is due to the use of the lateral line (Bleckmann, 1986; Bleckmann & Zelick, 2009). Individual *G. cotidianus* from lake populations possess lateral line systems adapted for sensitivity to changes in still waters as opposed to those from rivers (Vanderpham et al., 2013). Since the bullies used in this experiment came from

Lake Hayes, they would have the lake-morph lateral lines, potentially providing compensation for any visual impairment caused by *T. darbyi*, in this case. Another explanation could simply be that a net wielded by a human volunteer, although used in earlier studies of a similar nature (Seppälä et al., 2004), is not an accurate surrogate for a natural predator. Unfortunately, short of putting a live perch or eel in the arena and allowing them to feed on the bullies, this is as close to a "natural" interaction as we can achieve in a laboratory setting. Natural predators of *G. cotidianus* at Lake Hayes are either diving birds (e.g., grebes *Podiceps cristatus australis*), native fishes (e.g., eels) or introduced fishes (e.g., perch) all of which move through beneath the surface to hunt for prey, so using a net should accurately simulate a predation event by any of these potential threats.

Finally, the visual acuity of *G. cotidianus*, with and without parasites, is poorly understood. Vision in fishes is quite variable between and among species, thus making assumptions, however necessary, about how common bullies perceive visual stimuli is an inherent flaw. For instance, we do not know how important vision is for *G. cotidianus* when interacting with its environment. Many of the lakes in which *G. cotidianus* occurs are turbid (e.g., Lake Pukaki, Secchi depth = 0.6m) and/or deep (e.g., Lake Wanaka, 277 m) resulting in low visibility due to clarity, light conditions or both (Rowe, Graynoth, James, Taylor, & Hawke, 2003). As such, it is possible that other sensory systems, mentioned previously (i.e., olfaction and lateral line), compensate in low visibility conditions due to the wide variety of conditions in which the species occurs. We can, however, make inferences with regard to the visual ability of common bullies up to a point. For example, bullies darken in colour (i.e., light brown to black) in response to conspecifics (authors per. observation), especially males during the breeding season (McDowall, 1990). In fishes, darkening can be a way of creating contrast with the background space-light making themselves more visible to conspecifics (Guthrie, 1986; Kodric-Brown, 1998; Muntz, 1990). Therefore, it is apparent that visual stimuli are important for *G. cotidianus*, at least for some degree of conspecific communication, but the extent to which vision is used to identify and avoid predators, and whether that is influenced by eye-dwelling parasites, remains unclear.

In conclusion, we were unable to show a relationship between *T. darbyi* infection and the ability of its fish host, *G. cotidianus*, to react to and avoid simulated predatory threats. In part, our results could be due to our experimental conditions not recreating a realistic predation event such that reduced visual acuity would be apparent. The seeming lack of pathogenicity associated with *T. darbyi* combined with the ubiquity of infection in Lake Hayes bullies could also mask any deleterious side-effects related to avoiding predators. Future work investigating the impacts of parasites on predation rates of their hosts should attempt to create experimental conditions that are as realistic as possible. Unfortunately, this would likely require the use of actual predators which is not possible in the present case for ethical reasons. Furthermore, a better understanding of the behaviour of both the parasite (i.e. what is *T. darbyi* doing while in the eye) and the host (i.e. visual acuity of *G. cotidianus*) would allow for more sophisticated and directed experimental design.

ACKNOWLEDGEMENTS

We would like to thank the University of Otago for funding Brandon Ruehle via a postgraduate scholarship and associated student budget. Additionally, we want to thank Jolyn Chia, Lena Schallenberg, Vanitha Elangovan and the Evolutionary and Ecological Parasitology Laboratory at the University of Otago for technical assistance.

ORCID

Brandon Ruehle https://orcid.org/0000-0002-9898-3169

REFERENCES

Aeby, G. S. (2002). Trade-offs for the butterflyfish, *Chaetodon multicinctus*, when feeding on coral prey infected with trematode metacercariae. *Behavioural Ecology and Sociobiology*, *52*, 158–165. https://doi.org/10.1007/s00265-002-0490-2

Andersen, S. B., Gerritsma, S., Yusah, K. M., Mayntz, D., Hywel-Jones, N. L., Billen, J., … Hughes, D. P. (2009). The life of a dead ant: The expression of an adaptive extended phenotype. *American Naturalist*, *174*, 424–433. https://doi.org/10.1086/603640

Anderson, R. M., & May, R. M. (1978). Regulation and stability of host-parasite population interactions: I. regulator processes. *Journal of Animal Ecology*, *47*, 219–247.

Barber, I. (2007). Parasites, behaviour and welfare in fish. *Applied Animal Behaviour Science*, *104*, 251–264. https://doi.org/10.1016/j.applanim.2006.09.005

Barber, I., & Crompton, D. W. T. (1997a). The ecology of *Diplostomum phoxini* infections in two minnow (*Phoxinus phoxinus*) populations in Scotland. *Journal of Helminthology*, *71*, 189–196. https://doi.org/10.1017/S0022149X0001590X

Barber, I., & Crompton, D. W. T. (1997b). The distribution of the metacercariae of *Diplostomum phoxini* in the brain of minnows, *Phoxinus phoxinus*. *Folia Parasitologica*, *44*, 19–25.

Barber, I., Hoare, D., & Krause, J. (2000). Effects of parasites on fish behaviour: A review and evolutionary perspective. *Reviews in Fish Biology and Fisheries*, *10*, 131–165.

Barber, I., & Wright, H. A. (2006). Effects of parasites on fish behaviour: Interactions with host physiology. *Behaviour and Physiology of Fish*, *24*, 109–149. https://doi.org/10.1016/S1546-5098(05)24004-9

Blaso-Costa, I., Poulin, R., & Presswell, B. (2017). Morphological description and molecular analyses of *Tylodelphys* sp. (Trematoda: Diplostomidae) newly recorded from the freshwater fish *Gobiomorphus cotidianus* (common bully) in New Zealand. *Journal of Helminthology*, *91*, 332–345. https://doi.org/10.1017/S0022149X16000298

Bleckmann, H. (1986). Role of the lateral line in fish behaviour. In T. J. Pitcher (Ed.), *The behaviour of teleost fishes*. London, UK: Croom Helm Ltd.

Bleckmann, H., & Zelick, R. (2009). Lateral line system of fish. *Integrative Zoology*, *4*, 13–25. https://doi.org/10.1111/j.1749-4877.2008.00131.x

Burrough, R. J. (1978). The population biology of two species of eyefluke, *Diplostomum spathaceum* and *Tylodelphys clavata*, in roach and rudd. *Journal of Fish Biology*, *13*, 19–32. https://doi.org/10.1111/j.1095-8649.1978.tb03409.x

Bush, A. O., Lafferty, K. D., Motz, J. M., Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology*, *83*, 575–583. https://doi.org/10.2307/3284227

Carney, W. P. (1969). Behavioral and morphological changes in carpenter ants harbouring dicrocoeliid metacercariae. *American Midland Naturalist*, *82*, 605–611. https://doi.org/10.2307/2423801

Chibwana, F. D., Nkwengulila, G., Locke, S. A., McLaughlin, J. D., & Marcogliese, D. J. (2015). Completion of the life cycle of *Tylodelphys mashonense* (Sudarikov, 1971) (Digenea: Diplostomidae) with DNA barcodes and rDNA sequences. *Parasitology Research*, *114*, 3675–3682. https://doi.org/10.1007/S00436-015-4595-8

Chivers, D. P., & Smith, R. J. F. (1993). The role of olfaction in chemosensory based predator recognition in the fathead minnow, *Pimephales promelas*. *Journal of Chemical Ecology*, *19*, 623–633. https://doi.org/10.1007/BF00984997

Drago, F. B., & Lunaschi, L. I. (2008). Description of a new species of *Tylodelphys* (Digenea, Diplostomidae) in the wood stork, *Mycteria americana* (Aves, Ciconiidae) from Argentina. *Acta Parasitologica*, *53*, 263–267. https://doi.org/10.2478/s11686-008-0038-3

Fernald, R. D. (1990). The optical system of fishes. In R. H. Douglas, & M. B. A. Djamgoz (Eds.), *The visual system of fish*. London, UK: Chapman and Hall.

Flores, V., & Semenas, L. (2002). Infection patterns of *Tylodelphys barilochensis* and *T. crubensis* (Trematoda: Diplostomatidae) metacercariae in *Galaxias maculatus* (Osmeriformes: Galaxiidae) from two Patagonian lakes and observation on their geographical distribution in the Southern Andean Region, Argentina. *Journal of Parasitology*, *88*, 1135–1139. https://doi.org/10.1645/0022-3395(2002)088[1135:IPOTBA]2.0.CO;2

Fredensborg, B. L., & Longoria, A. N. (2012). Increased surfacing behaviour in longnose killifish infected by brain-encysting trematode. *Journal of Parasitology*, *98*, 899–903. https://doi.org/10.1645/GE-3170.1

Galaktionov, K. V., & Dobrovolskij, A. A. (2003). *The biology and evolution of trematodes*. Dordrecht, the Netherlands: Kluwer Academic Publishers.

Garcia-Varela, M., Sereno-Uribe, A. L., Pinacho-Pinacho, C. D., Hernandez-Cruz, E., & Perez-Ponce de Leon, G. (2016). An integrative taxonomic study reveals a new species of *Tylodelphys* Diesing, 1950 (Digenea: Diplostomidae) in central and northern Mexico. *Journal of Helminthology*, *90*, 668–679. https://doi.org/10.1017/S0022149X15000917

Guthrie, D. M. (1986). Role of vision in fish behaviour. In T. J. Pitcher (Ed.), *The behaviour of teleost fishes*. London, UK: Croom Helm Ltd.

Hammond-Tooke, C. A., Nakagawa, S., & Poulin, R. (2012). Parasitism and behavioural syndromes in the fish *Gobiomorphus cotidianus*. *Behaviour*, *149*, 601–622. https://doi.org/10.1163/156853912X648903

Hara, T. J. (1986). Role of olfaction in fish behaviour. In T. J. Pitcher (Ed.), *The behaviour of teleost fishes*. London, UK: Croom Helm Ltd.

Holmes, J. C., & Bethel, W. M. (1972). Modification of intermediate host behaviour by parasites. *Zoological Journal of the Linnaean Society*, *51*, 123–149.

Karvonen, A., Paukku, A., & Seppälä, O. (2005). Resistance against eye flukes: naïve versus previously infected fish. *Parasitology Research*, *95*, 55–59. https://doi.org/10.1007/s00436-004-1246-x

Karvonen, A., Seppälä, O., & Valtonen, T. (2004). Eye fluke-induced cataract formation in fish: Quantitative analysis using an ophthalmological microscope. *Parasitology*, *129*, 473–478. https://doi.org/10.1017/S0031182004006006

Kennedy, C. R. (1987). Long-term stability in the population levels of the eyefluke *Tylodelphys podicipina* (Digenea: Diplostomatidae) in perch. *Journal of Fish Biology*, *31*, 571–581. https://doi.org/10.1111/j.1095-8649.1987.tb05259.x

Kodric-Brown, A. (1998). Sexual dichromatism and temporary color changes in the reproduction of fishes. *American Zoologist*, *38*, 70–81. https://doi.org/10.1093/icb/38.1.70

Kuris, A. M., Blaustein, A. R., & Alio, J. J. (1980). Hosts as islands. *American Naturalist*, *116*, 570–586. https://doi.org/10.1086/283647

Lafferty, K. (1999). The evolution of trophic transmission. *Parasitology Today*, *15*, 111–115. https://doi.org/10.1016/S0169-4758(99)01397-6

Lafferty, K., & Morris, A. K. (1996). Altered behaviour of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology*, *77*, 1390–1397. https://doi.org/10.2307/2265536

Martin, W. E. (1950). *Euhaplorchis californiensis* n.g., n. sp., Heterophyidae, Trematoda, with notes on its life-cycle. *Transactions of the American Microscopical Society*, *69*, 194–209. https://doi.org/10.2307/3223410

McDowall, R. M. (1990). *New Zealand freshwater fishes: a natural history and guide*. Auckland, New Zealand: Heinemann Reed MAF Publishing Group.

Michel, C., Hicks, B. J., Stolting, K. N., Clarke, A. C., Stevens, M. I., Tana, R., … van den Heuval, M. R. (2008). Distinct migratory and non-migratory ecotypes of an endemic New Zealand eleotrid (*Gobiomorphus cotidianus*) – implications for incipient speciation in island freshwater species. *BMC Evolutionary Biology*, *8*, https://doi.org/10.1186/1471-2148-8-49

Moore, J. (2002). *Parasites and the behavior of animals*. Oxford, UK: Oxford University Press.

Moore, J. (2013). An overview of parasite-induced behavioural alterations – and some lessons from bats. *Journal of Experimental Biology*, *216*, 11–17. https://doi.org/10.1242/jeb.074088

Munoz, J. C. V., Staaks, G., & Knopf, K. (2017). The eye fluke *Tylodelphys clavata* affects prey detection and intraspecific competition of European perch (*Perca fluviatilis*). *Parasitology Research*, *116*, 2561–2567. https://doi.org/10.1007/s00436-017-5564-1

Muntz, W. R. A. (1990). Stimulus, environment and vision in fishes. In R. H. Douglas, & M. B. A. Djamgoz (Eds.), *The visual system of fish*. London, UK: Chapman and Hall.

Poulin, R. (2000). Variation in the intraspecific relationship between fish length and intensity of parasitic infection: Biological and statistical causes. *Journal of Fish Biology*, *56*, 123–137. https://doi.org/10.1111/j.1095-8649.2000.tb02090.x

Poulin, R. (2010). Parasite manipulation of host behaviour: An update on frequently asked questions. In H. J. Brockmann (Ed.), *Advances in the study of behavior* (pp. 151–186). New York, NY: Elsevier Inc., Academic Press.

Poulin, R., & George-Nascimento, M. (2007). The scaling of total parasite biomass with host body mass. *International Journal for Parasitology*, *37*, 359–364. https://doi.org/10.1016/j.ijpara.2006.11.009

Presswell, B., & Blasco-Costa, I. (2019). Description of *Tylodelphys darbyi* n. sp. (Trematoda: Diplostomidae) from the threatened Australasian crested grebe (*Podiceps cristatus australis*, Gould 1844) and linking of its life-cycle stages. *Journal of Helminthology*, 1–8. https://doi.org/10.1017/S0022149X19000142

Rowe, D., Graynoth, E., James, G., Taylor, M., & Hawke, L. (2003). Influence of turbidity and fluctuating water levels on the abundance and depth distribution of small, benthic fish in New Zealand alpine lakes. *Ecology of Freshwater Fish*, *12*, 216–227. https://doi.org/10.1034/j.1600-0633.2003.00024.x

Ruehle, B. P., Herrmann, K. K., & Higgins, C. L. (2017). Helminth parasite assemblages in two cyprinids with different life history strategies. *Aquatic Ecology*, *51*, 247–256. https://doi.org/10.1007/s10452-017-9614-7

Seppälä, O., Karvonen, A., & Valtonen, E. T. (2011). Eye fluke-induced cataracts in natural fish populations is there potential for host manipulation? *Parasitology*, *138*, 209–214. https://doi.org/10.1017/S0031182010001228

Seppälä, O., Karvonen, A., & Valtonen, T. (2004). Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke-fish interaction. *Animal Behaviour*, *68*, 257–263. https://doi.org/10.1016/j.anbehav.2003.10.021

Seppälä, O., Karvonen, A., & Valtonen, T. (2005). Manipulation of fish host by eye flukes in relation to cataract formation and parasite infectivity. *Animal Behaviour*, *70*, 889–894. https://doi.org/10.1016/j.anbehav.2005.01.020

Seppälä, O., Karvonen, A., & Valtonen, T. (2008). Shoaling behaviour of fish under parasitism and predation risk. *Animal Behaviour*, *75*, 145–150. https://doi.org/10.1016/j.anbehav.2007.04.022

Shariff, M., Richards, R. H., & Sommerville, C. (1980). The histopathology of acute and chronic infections of rainbow trout Salmo gairdneri Richardson with eyeflukes, Diplostomum spp. *Journal of Fish Diseases*, *3*, 455–465.

Stumbo, A. D., & Poulin, R. (2016). Possible mechanism of host manipulation resulting from a diel behaviour pattern of eye-dwelling parasites? *Parasitology*, *143*, 1261–1267. https://doi.org/10.1017/S0031182016000810

Vanderpham, J. P., Nakagawa, S., & Closs, G. P. (2013). Habitat-related patterns in phenotypic variation in a New Zealand freshwater generalist fish, and comparisons with a closely related specialist. *Freshwater Biology*, *58*, 396–408. https://doi.org/10.1111/fwb.12067

Vanderpham, J. P., Nakagawa, S., & Clossy, G. P. (2012). Diel variation in use of cover and feeding activity of a benthic freshwater fish in response to olfactory cues of a diurnal predator. *Environmental Biology of Fishes*, *93*, 547–556. https://doi.org/10.1007/s10641-011-9949-1

Wesolowska, W., & Wesolowski, T. (2014). Do *Leucochloridium* sporocysts manipulate the behavior of their snail hosts? *Journal of Zoology*, *292*, 151–155. https://doi.org/10.1111/jzo.12094

How to cite this article: Ruehle B, Poulin R. No impact of a presumed manipulative parasite on the responses and susceptibility of fish to simulated predation. *Ethology*. 2019;125:745–754. https://doi.org/10.1111/eth.12927