



# Risky business: influence of eye flukes on use of risky microhabitats and conspicuousness of a fish host

Brandon Ruehle<sup>1</sup> · Robert Poulin<sup>1</sup>

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## Abstract

A prerequisite for a parasitic manipulation to be considered adaptive is that it confers a fitness benefit to the parasite, such as increased transmission to another host. These manipulations can involve alterations to a wide range of host phenotypic traits, including microhabitat choice. Eye flukes of the trematode family Diplostomidae use fish as intermediate hosts and must be transmitted by predation to a piscivorous bird. In New Zealand, the diplostomid *Tylodelphys darbyi* infects the eyes of a widespread endemic freshwater fish, the common bully *Gobiomorphus cotidianus*. Within the eye, *T. darbyi* metacercariae achieve large sizes and move freely about the aqueous and vitreous humors of the eye. We hypothesized that higher intensities of *T. darbyi* would (i) cause bullies to show increased activity and spend more time moving about in open space (i.e., more conspicuous, risky microhabitat) and (ii) reduce their ability to compete for shelter with fish harboring lower infection levels. Our experiments showed that heavily infected fish were more active and spent more time in the open, although the effect was age-dependent, with immature fish displaying decreases in activity and time spent in the open with increasing intensities of infection. We also demonstrated that heavily infected female bullies have a lower probability of using shelter, but males show the opposite pattern. It is possible that using more risky microhabitats increases the likelihood of the fish being eaten by the parasite's predatory avian definitive hosts. However, our findings indicate that age- and sex-dependent effects call for a more nuanced interpretation.

**Keywords** *Gobiomorphus cotidianus* · Host manipulation · Eye fluke · Diplostomid · Microhabitat · Host-parasite interactions

## Introduction

The prerequisite for any parasitic manipulation to be considered adaptive is that it confers a fitness benefit to the parasite. Different types of host manipulations are expected based on the life cycle and transmission mode of the parasite (Poulin 2010). Thus, specific kinds of host manipulation are associated with parasites that require their hosts to move to a new area (e.g., nematomorphs, *Cordyceps* fungi) to allow or enhance the dispersal of the parasite's propagules (Andersen et al. 2009; Poulin 2010; Bolek et al. 2013), and other types of manipulations would be expected for vector-borne diseases

and parasitoids. However, the best-studied are those involving parasites that are trophically transmitted (Lafferty 1999; Poulin 2010). Parasite-increased trophic transmission occurs in parasite taxa that have complex, multi-host life cycles in which one host must be ingested by another (Lafferty 1999; Moore 2002, 2013; Poulin 2010). Some parasites accomplish this by physically changing the appearance (e.g., color and/or morphology) of the host to attract predators or impair escape responses (Poinar and Yanoviak 2008; Poulin 2010; Moore 2013; Wesolowska and Wesolowski 2014). Other parasites cause the host to put itself at greater risk of predation by increasing its activity levels, or changing its use of microhabitats or shelter (Holmes and Bethel 1972; Bethel and Holmes 1973; Moore 1983, 1984; Moore and Lasswell 1986).

Aquatic ecosystems harbor a variety of parasites with complex lifecycles (e.g., cestodes, trematodes) (Barber and Poulin 2002). Fish act as intermediate hosts to a wide range of parasites in natural ecosystems and as such are at risk of behavioral alterations (Barber et al. 2000; Barber and Poulin 2002; Barber and Wright 2006). In some cases, parasites impact

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✉ Brandon Ruehle  
brandon.ruehle90@gmail.com

<sup>1</sup> Department of Zoology, University of Otago, 340 Great King Street, Dunedin 9016, New Zealand

the host by changing time budgets and habitat choice due to physiological demands of infection (Brassard et al. 1982; Barber and Wright 2006). For example, sticklebacks, *Gasterosteus aculeatus*, infected with the cestode *Schistocephalus solidus* have increased oxygen demands and as a result spend more time in shallower waters and nearer the surface (Lester 1971; Barber and Wright 2006; Poulin 2018). Lester (1971) suggested anecdotally that infected sticklebacks were at greater risk of predation by birds due to these behavioral changes. *Schistocephalus solidus* infection also causes sticklebacks to avoid shoaling with uninfected conspecifics, potentially, directly or indirectly, increasing predation risk due to higher vulnerability (Barber et al. 1995). Trematode parasites of fish can also impact the behavior of the host by invading sensory organs or central nervous system (e.g., the brain, the eyes) (Barber and Crompton 1997a, b; Barber and Wright 2006). For instance, metacercariae of *Euhaplorchis* spp. infect the brains of killifish, *Fundulus* spp., causing the host to perform conspicuous movements (e.g., flashing, surfacing, contorting) (Lafferty and Morris 1996; Fredensborg and Longoria 2012). The killifish in this case serves as an intermediate host for the parasite and the altered swimming patterns result in greater rates of transmission to birds, the definitive host (Lafferty and Morris 1996; Fredensborg and Longoria 2012).

A group of trematodes often studied for their effects on fish behavior are diplostomid eye flukes. *Diplostomum* spp. can be found as metacercariae in the lenses of a variety of fish where they generate cataracts due to the release of waste and as such may have multiple effects on the host (Owen et al. 1993; Karvonen et al. 2004; Seppälä et al. 2004, 2005a; Kalbe and Kurtz 2006; Seppälä et al. 2008; Voutilainen et al. 2008, 2010; Ubels et al. 2018). Rainbow trout, *Oncorhynchus mykiss*, infected with *Diplostomum spathaceum* engage in behaviors that put them at greater risk of predation. For example, those harboring the parasite do not shoal with uninfected fish and are more vulnerable to a simulated predator (Seppälä et al. 2004, 2008). Additionally, when provided with the choice of a dark or light background, infected trout spent more time over the light area which made them visually more conspicuous (Seppälä et al. 2005b). Closely related eye flukes, *Tylodelphys* spp., are also found as metacercariae in the eyes of fish, but these parasites are comparatively understudied for their behavioral impacts (e.g., Muñoz et al. 2017, 2019; Ruehle and Poulin 2019).

The recently described *Tylodelphys darbyi* infects the eyes of a native fish, the common bully *Gobiomorphus cotidianus*, on the South Island of New Zealand (Stumbo and Poulin 2016; Blasco-Costa et al. 2017; Presswell and Blasco-Costa 2019). In the eyes of *G. cotidianus*, metacercariae of *T. darbyi* move freely within the fluid parts (i.e., the aqueous and vitreous humors) of the eye, can be > 1 mm in length, and commonly reach intensities of > 10 per eye, but, unlike *Diplostomum* spp.,

do not cause noticeable pathology (Stumbo and Poulin 2016; Blasco-Costa et al. 2017; Presswell and Blasco-Costa 2019). The definitive host of *T. darbyi* is the Australasian great-crested grebe *Podiceps cristatus australis*, a diving bird that actively pursues fish under water. Common bullies are benthic, unlike sticklebacks and trout, and as a result do not form proper shoals. Instead, they use underwater structures (e.g., rocks, logs) as shelter and can form loose aggregations if enough structure is provided (McDowall 1990; per authors' observation). Few studies have investigated this host-parasite system (e.g., Stumbo and Poulin 2016; Ruehle and Poulin 2019) and none has investigated how infection could impact the bully's movement (e.g., conspicuous behavior, microhabitat/shelter use) within the environment. Therefore, the objectives of the present study are to determine if *T. darbyi* infection (1) causes common bully to be more conspicuous due to increased activity levels and (2) impacts the choice of microhabitat (i.e., shelter or not) when alone and when in groups. We hypothesize that higher intensities of *T. darbyi* will cause bullies to show increased activity and spend more time moving about in open space (i.e., more conspicuous, risky microhabitat) and will reduce their ability to compete for shelter with fish harboring lower infection levels.

## Materials and methods

### Collection, housing, and tagging

Common bullies *Gobiomorphus cotidianus* were collected from Lake Hayes, located in the Southern Alps of New Zealand's South Island 14.5 km from Queenstown. Fish were caught using minnow traps placed along the lake margins overnight and recovered the following morning. After collection, fish were transported back to the University of Otago in 25-L ice chests provided with aeration. Holding tanks were provided with filtration and aeration and filled with a solution of 1/3 saltwater and 2/3 freshwater to prevent fungal growth. The tanks were lit from above on an automatic 12-h day/night cycle, fish fed commercial fish pellets ad libitum, and water kept at room temperature (i.e., 20 °C). Tank sizes differed between the two experiments (see below) but stocking densities were kept at < 2 fish/L and fish were provided with enough shelter (e.g., 10-cm PVC pipe pieces) such that each could establish an individual territory. Following a 72-h acclimation period, fish were anesthetized (5 min in MS-222 solution, 1 mg/L) and tagged with a unique, visible, 2-mm-long elastomer implant tag (Northwest Marine Technologies Inc.) for identification. To minimize observer bias, blinded methods were used to record behaviors in the following experiments. Indeed, because the infection status of each individual fish can only be determined through dissections after the behavioral observations, all behavior measurements were done with the

observer completely unaware of whether a fish was infected, and if so by how many parasites.

### Experiment 1: individual microhabitat use and activity level

Fish ( $n = 35$ ) were collected in summer 2018 and held in groups of 3 in 14-L tanks (31.5 cm × 19 cm × 14.5 cm). The testing arena (36 L; 44 cm × 27.5 cm × 29.5 cm) was split into two sides, one provided with light and the other without; following the procedure in Ruehle and Poulin (2019), the dark side of the arena represents shelter (e.g., an overhang, rock). A 100-W desk lamp was used as the light source, all other lights in the room were shut off, and a divider (i.e., cardboard covered in black plastic) was used to direct the light to one side and minimize penetration into the other. Individual fish were introduced to the lit side of the arena in a glass jar for a 30-min acclimation period, after which the jar was gently lifted releasing the fish for a 30-min video session. All trials were recorded using a GoPro Black5 camera controlled remotely by the observer. During video observation, the amount of time (min) each fish spent in the lighted area was recorded as well as the number of times the individual crossed from one side to the other as a measure of activity. Following a 48-h rest period, fish were tested a second time in a different but identical arena in which the light and dark sides were switched.

### Experiment 2: group microhabitat use

Individuals ( $n = 30$ ) for this experiment were collected in spring 2018 and held in 6 size-matched groups in 36-L tanks (44 cm × 27.5 cm × 29.5 cm). Groups initially consisted of 5 or 6 fish, based on size classes, but due to mortality during captivity, one group consisted of 4 fish only. Two identical testing arenas (54 cm × 80 cm × 28 cm) were divided into quadrants, 2 with shelter and 2 without, and provided with a layer of aquarium gravel. Arenas in this experiment are larger than in experiment 1 due to testing multiple fish together and the potential territorial nature of *G. cotidianus*. Shelter quadrants were oriented diagonal to each other and were equipped with 2 “shelters” consisting of PVC tubes (diameter = 4 cm, length = 10 cm) attached to 10 × 10-cm ceramic tiles. A group of fish was introduced to the center of the arena and allowed 1 h of acclimation before the first observation, at which point the location of each fish (shelter quadrant scored as 1, no shelter quadrant as 0) was recorded. Four further observations were made, each with an hour in between, for a total of 5. Each group was tested twice with 48 h in between trials.

### Parasite counts

All fish were euthanized by a combination of an overdose of MS-222 (10 mg/L) and spinal severance as well as measured

for standard length (SL) and weighed (TM). Each eye was removed and examined for *Tyloodelphys darbyi* infection separately. Considering *T. darbyi* is found in the humors of the eye, we focused our efforts on this area, but the lens and retina were also examined. Common bullies are hosts to several other parasites in addition to *T. darbyi*; therefore, we examined the rest of the fish for infection as well. Three trematode taxa, *Apatemon* sp., *Telogaster opisthorchis*, and *Stegodexamene anguillae*, are common bully parasites. They are found encysted as metacercariae in and around various organs (e.g., gonads and liver), in the connective tissues in the body cavity, and in muscle tissues. A nematode, *Eustrongylides* sp., can also be found in the body cavity of common bully and so their presence was noted as well. We calculated, in addition to infection intensity, the prevalence (proportion of individual fish infected) and mean intensity of infection (mean number of parasite individuals per infected fish) for all parasite taxa recovered.

### Statistical analysis

We performed all statistical analyses and generated figures using the *lme4*, *lmerTest*, *visreg* (Breheny and Burchett 2017), and *ggplot2* packages in the program R (version 3.5.3; R Core Development Team, 2019). For the shelter and activity experiment, we ran mixed effects models (GLMM, *glmer* function, and LMM, *lmer* function) with fish ID as the random factor to account for each fish being used in two trials. The first GLMM was fitted with the Poisson distribution and used the number of crosses (from one side of the tank to the other) as the response variable, and the other analysis was run as a standard Gaussian LMM with time spent in the open (i.e., the lit side) as the response. Both models used *Tyloodelphys darbyi* intensity, SL, and sexual maturity (mature vs immature) of the individual fish as predictor variables.

A single GLMM was used for the second experiment (group microhabitat use) with fish ID and group number as random factors, to account for the 5 observations per trial and two replicate trials. We ran the model using the binomial distribution with the choice of quadrant (shelter = 1, no shelter = 0) as the response variable and *T. darbyi* intensity, SL, and sex of each fish as predictors. The first two models used maturity rather than sex as a predictor because for the summer 2018 collection, there were immature individuals (i.e., lacking developed gonads) which prevented differentiation of males from females.

Intensity of the trematode *Apatemon* sp. was used originally as a predictor in all models, as it was by far the most abundant taxa found (see Results) for both experiments, but was later excluded due to the variable generating scale errors, even after scaling the predictors, as well as causing the models to fail to converge with the model intercept, which can lead to false positives. This issue remains for any model iteration that

includes *Apatemon* sp. Therefore, to test the influence of this parasite, we performed Spearman's rank correlations for the first experiment (i.e., number of crosses and time in the open) and a Mann-Whitney-Wilcoxon test for the second (i.e., use of shelter) with *Apatemon* sp. intensity as the dependent variable. Other parasite taxa (i.e., *T. opisthorchis*, *S. anguillae*, and *Eustrongylides* sp.) were not used for analyses due to their comparatively low prevalences and intensities (see Results). The first two models used maturity rather than sex as a predictor because for the summer 2018 collection, there were numerous immature individuals (i.e., lacking developed gonads) which prevented differentiation of males from females.

## Results

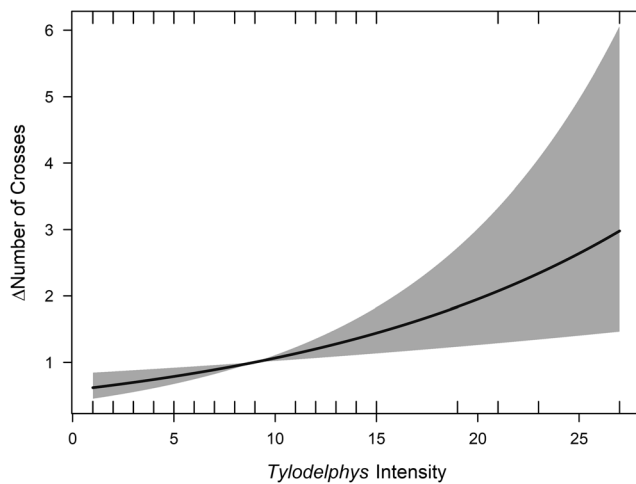
We recovered 5 parasite taxa from 65 *Gobiomorphus cotidianus* over the course of our two experiments (Table 1). All fish were infected with *Tylodelphys darbyi* with an average of ~18 worms per individual (Table 1). Most *T. darbyi* metacercariae were found in the aqueous and vitreous humors in the eye, but in the spring collection, a total of 13 worms were found in the braincases of 8 individuals. Three other trematode taxa, *Apatemon* sp., *Telogaster opisthorchis*, and *Stegodexamene anguillae*, were recovered from both spring and summer collections (Table 1). All three taxa were found in

the muscles and connective tissues with *T. opisthorchis* and *S. anguillae* also occurring in and on some organs (e.g., liver, gonads). *Apatemon* sp. was found in high intensities in all fish sampled, but the other taxa occurred in much lower numbers and prevalences (Table 1). The nematode *Eustrongylides* sp. was recovered as a larva and was always associated with tissues surrounding the alimentary canal.

In the first experiment on individual fish, *Tylodelphys darbyi* intensity significantly predicted the number of times the bullies crossed from one side of the arena to the other, with the more heavily infected fish showing greater activity ( $z = -3.64$ ,  $df_{\text{resid}} = 62$ ,  $P < 0.001$ ; Fig. 1). On their own, SL and maturity (i.e., mature vs immature) of the fish did not predict the number of crosses, but there was a significant two-way interaction between these two predictors ( $z = -2.29$ ,  $df_{\text{resid}} = 62$ ,  $P = 0.02$ ). There were further significant two-way interactions between *T. darbyi* intensity and SL ( $z = -2.60$ ,  $df_{\text{resid}} = 62$ ,  $P = 0.009$ ) as well as with the maturity of the fish ( $z = 4.37$ ,  $df_{\text{resid}} = 62$ ,  $P < 0.001$ ; Fig. 2). The amount of time a fish spent on the lit side of the arena was significantly predicted by *T. darbyi* intensity ( $t = -2.40$ ,  $df_{\text{resid}} = 28$ ,  $P = 0.02$ ; Fig. 3) but not SL ( $t = 0.74$ ,  $df_{\text{resid}} = 28$ ,  $P = 0.46$ ) or maturity ( $t = -0.35$ ,  $df_{\text{resid}} = 28$ ,  $P = 0.73$ ). A two-way interaction between *T. darbyi* intensity and maturity significantly influenced the time spent in the light side ( $t = 2.46$ ,  $df_{\text{resid}} = 28$ ,  $P = 0.02$ ; Fig. 4), but the interaction between *T. darbyi* and SL did not

**Table 1** Percent prevalence, mean intensity, and range of intensity for all parasite taxa recovered for each collection as well as overall

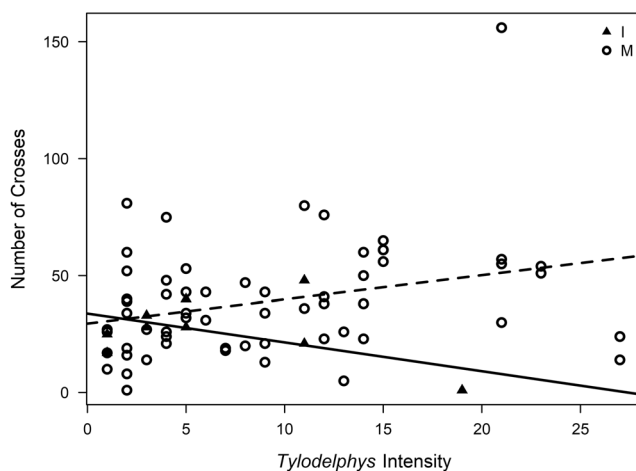
Collection	Helminth taxa	Prevalence (%)	Mean intensity	Intensity range	
				Min	Max
Summer <i>n</i> = 35	<i>Tylodelphys darbyi</i>	100.0	8.9	1	27
	<i>Apatemon</i> sp.	100.0	254.4	85	560
	<i>Telogaster opisthorchis</i>	40.0	3.6	0	11
	<i>Stegodexamene anguillae</i>	17.1	1.8	0	6
	<i>Eustrongylides</i> sp.	5.7	1.5	0	2
Spring <i>n</i> = 30	<i>Tylodelphys darbyi</i>	100.0	28.7	5	67
	<i>Apatemon</i> sp.	100.0	233.4	62	469
	<i>Telogaster opisthorchis</i>	83.3	7.2	0	70
	<i>Stegodexamene anguillae</i>	86.7	5.8	0	25
	<i>Eustrongylides</i> sp.	30.0	1.3	0	2
Overall <i>n</i> = 65	<i>Tylodelphys darbyi</i>	100.0	18.1	1	67
	<i>Apatemon</i> sp.	100.0	244.7	62	560
	<i>Telogaster opisthorchis</i>	60.0	5.9	0	70
	<i>Stegodexamene anguillae</i>	49.2	5.1	0	25
	<i>Eustrongylides</i> sp.	16.9	1.4	0	2



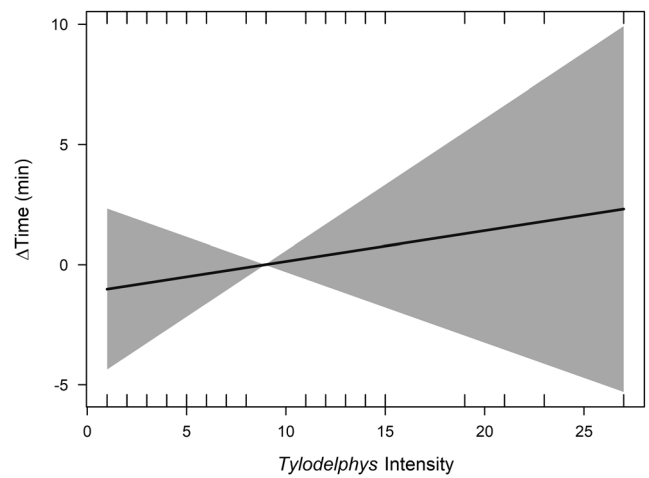
**Fig. 1** Relationship between *T. darbyi* intensity and the number of times a bully crossed the arena. The shaded areas are confidence intervals with alpha set at 0.05 and have zero width at the mean value of *T. darbyi* intensity. The y-axis indicates how many more or fewer crosses are expected for a fish with more or fewer parasites than the mean intensity. Rug marks show partial residuals with positive values on the top and negative on the bottom

( $t = 0.06$ ,  $df_{resid} = 28$ ,  $P = 0.95$ ). There was no interaction between SL and maturity of the fish ( $t = -1.01$ ,  $df_{resid} = 28$ ,  $P = 0.32$ ). Finally, there was no relationship between *Apatemon* sp. intensity and either activity level ( $r_s = 0.18$ ,  $P = 0.13$ ) or time in the open ( $r_s = -0.18$ ,  $P = 0.14$ ).

In the second experiment using groups of fish, shelter use of the bullies was predicted by *T. darbyi* intensity ( $z = -2.39$ ,  $df_{resid} = 291$ ,  $P = 0.02$ ), but not by SL ( $z = -1.20$ ,  $df_{resid} = 291$ ,  $P = 0.23$ ) or sex of the fish ( $z = 0.54$ ,  $df_{resid} = 291$ ,  $P = 0.59$ ). Finally, the interaction between sex and *T. darbyi* intensity significantly affected how the fish used shelter ( $z = 1.98$ ,  $df_{resid} = 291$ ,  $P = 0.048$ ; Fig. 5), with increasing *T. darbyi* intensities having opposite effects on male and female fish. There



**Fig. 2** Scatterplot showing relationship between *T. darbyi* intensity and number of arena crosses. The solid line shows the negative relationship for immature individuals (I) and the dashed line represents mature fish (M)

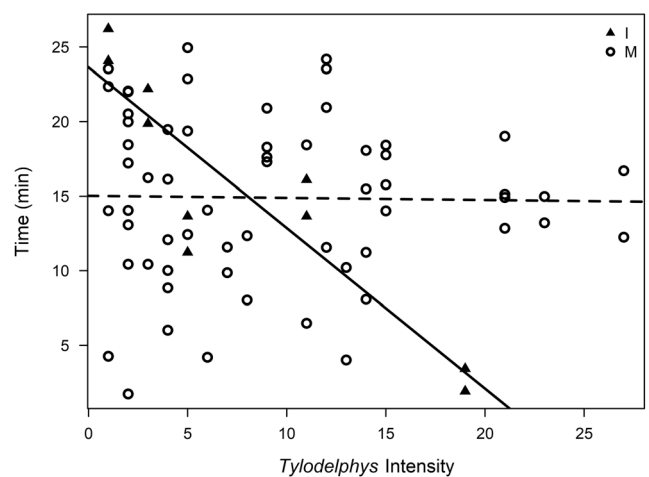


**Fig. 3** Relationship between *T. darbyi* intensity and the time in minutes a bully spent on the lit side of the arena. The shaded areas are confidence intervals with alpha set at 0.05 and have zero width at the mean value of *T. darbyi* intensity. The y-axis indicates how much more or less time in the lit side is expected for a fish with more or fewer parasites than the mean intensity. Rug marks show partial residuals with positive values on the top and negative on the bottom

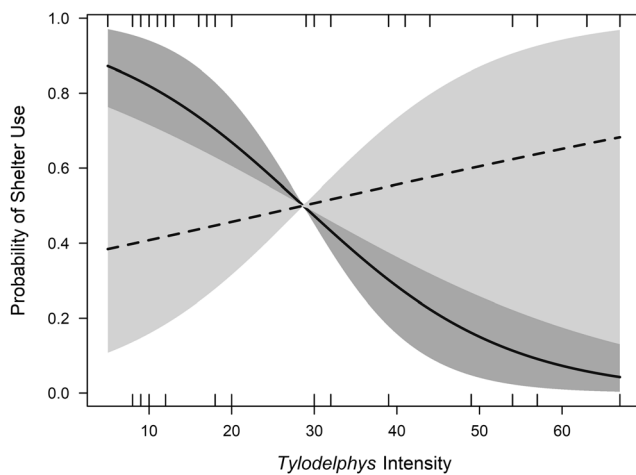
was also no difference in *Apatemon* sp. intensity between fish that used shelter and those that did not ( $W = 5895$ ,  $P = 0.17$ ).

### Discussion

Parasites have the ability to alter the behavior of their host, causing them to be more conspicuous and choose risky microhabitats (Holmes and Bethel 1972; Bethel and Holmes 1973; Moore 1983; Moore 1984; Moore and Lasswell 1986; Lafferty and Morris 1996; Fredensborg and Longoria 2012). We have shown that the intensity of *Tyloodelphys darbyi*



**Fig. 4** Scatterplot showing relationship between *T. darbyi* intensity and the time in minutes a bully spent on the lit side of the tank. Different symbols indicate immature (I) and mature (M) individuals. The solid line shows the negative relationship for immature individuals, and the dashed line represents mature fish and is added for contrast



**Fig. 5** Relationship between *T. darbyi* intensity and the probability of a bully using a shelter quadrant. Rug marks show partial residuals with positive values on the top and negative on the bottom. Shaded area is the confidence band with alpha set at 0.05. The lines represent the relationship for males (solid) and females (dashed)

infection may have some impact on the activity level and shelter use of *Gobiomorphus cotidianus* singly and in groups. Our first experiment showed that as *T. darbyi* intensity increases fish are more active (i.e., cross the center of the arena more often; Fig. 1) and spend more time in the open (i.e., the lit side of the arena; Fig. 3), potentially increasing their conspicuousness to visually searching predators. However, the confidence bands for the relationships in both Figs. 1 and 3 indicate that the effect of high infection intensity is variable and likely modulated by other factors. We also found that immature bullies were less active and spent less time in the open with increasing numbers of *T. darbyi* than did mature fish (Fig. 4). In the group shelter experiment, we observed that the probability of females using a shelter quadrant decreased with increasing numbers of *T. darbyi*, while for males, it increased (Fig. 5). A previous study demonstrated that eye flukes can have an impact on the microhabitat (i.e., light vs dark background and associated color change) choice of their fish hosts (Seppälä et al. 2005b). The authors experimentally infected farmed, juvenile rainbow trout *Oncorhynchus mykiss* with *Diplostomum spathaceum*, a parasite known to cause obvious pathology (i.e., cataracts). In contrast, our study examines a native fish harboring natural infections with a parasite that has no known pathological impact on the host (Stumbo and Poulin 2016).

Common bullies, as benthic fish, use structure (e.g., rocks, woody debris) for protection from predators, so any stressor that causes the fish to abandon that shelter should increase the risk of being captured (Rahel and Stein 1988). In other fish species that actively swim about the water column, conspicuous behavior manifests itself as altered movement patterns such as darting, contorting, and/or surfacing (Lafferty and Morris 1996; Fredensborg and Longoria 2012). However,

increased conspicuousness in bullies might simply involve leaving a protective shelter and spending more time moving about in the open, potentially resulting in the fish being captured by *T. darbyi*'s avian definitive host. Actual risk of predation was not tested in this study however; therefore, there may be explanations other than parasitic manipulation. Barber et al. (1995) suggest that infected sticklebacks were less likely to shoal with uninfected conspecifics due to a greater need to forage for food, particularly away from competition. It is possible that higher intensities of *T. darbyi* cause the fish to spend more time exploring the environment for resources due to their vision being impaired. In such a case, increased risk of predation would be a coincidental side effect of infection rather than direct manipulation by the parasite (Poulin 2010).

We have shown that higher intensities of *T. darbyi* may coincide with fish being more active. However, rather than a manipulation, or other impact of infection, these individuals could have been innately more active than the other fish in the sample, in which case higher intensities of infection might be a consequence of behavior, and not their cause. It has been shown that fish that spend more time moving about their environment risk encountering infective stages of parasites more often than others in the population (Poulin et al. 1991; Wilson et al. 1993). Poulin et al. (1991) demonstrated this with ectoparasitic copepods infecting mobile brook trout fry at a higher rate than those that remained stationary. Similarly, the life stage of *T. darbyi* infective to bullies, cercariae, freely move about the water body in search of a host (Selbach and Poulin 2018); therefore, more active individuals could have simply contacted and acquired more parasites for this reason. Contrary to this explanation, Koprivnikar et al. (2011) found that the most active and exploratory tadpoles in their study had lower levels of trematode infection. While this does not fit with what we observed for mature bullies, it matches the patterns seen in the immature fish, in which the least active individuals harbored higher infection intensities. Perhaps active immature individuals are in better physical condition and as such able to fend off infection and its effects, than the other fish (Koolhaas 2008; Koprivnikar et al. 2011).

The bullies for these experiments were collected at different times of the year, and as such, this could have had an impact on the outcomes. Mean intensity of *T. darbyi* was almost 4-fold higher in the spring collection than in the summer (Table 1;  $t = -5.28$ ,  $df = 35.68$ ,  $P < 0.001$ ). Higher numbers of parasites in their eyes could have a greater influence on the fish's behavior. However, since we considered both collections separately, and both contained a range of infection levels from low to high (Table 1), it is unlikely that the disparity greatly impacted our findings. A seasonal difference in the behavior of the bullies could be relevant, however, as we found that there may be difference between males and females in the group shelter experiment. The breeding season for common bully generally starts in October (i.e., entering Austral

summer; McDowall 1990), and our experiment took place in early November. Male common bullies usually develop a distinctive mating coloration (e.g., turn black) and guard an area (i.e., structure or shelter) in which they entice a female to lay her eggs (Stephens 1982; McDowall 1990). Therefore, a possible explanation for the observed greater probability (Fig. 5) of males using shelter could be their territorial behavior, while conversely females need to search out a mate. Previous work has shown that there can be differences between sexes in both the level of infection and behavioral effects of the parasites (Richards and van Oosterhout 2010; Duneau and Ebert 2012; Stephenson et al. 2015; Tadiri et al. 2016). This observed difference between the sexes may result from intrinsic biological differences between the sexes and is superimposed on the effect of parasite infection, which seems to exacerbate the wrong behavior in both sexes. Males with high intensities of *T. darbyi* eye flukes appear to spend less time in a shelter, possibly compensating for impaired vision by allocating more time to searching for food. In contrast, although the effects in females was generally weaker and more variable than in males, females with high intensities of eye flukes seem to be more likely to be near a shelter, perhaps shifting their trade-off between mate searching and predator avoidance.

In trematodes whose metacercariae inhabit fish eyes, natural selection may have favored location in the eye as a means to evade the host's immune response, and not directly for the function of manipulating host behavior (Locke et al. 2010). Whatever the evolutionary reasons for their presence in fish eyes, there may be consequences for fish vision and behavior. Here, we demonstrate that the eye fluke *T. darbyi* may impact the microhabitat choice and activity of common bully; this is the first study showing potential behavioral changes associated with this parasite. It is possible that using more risky microhabitats increases the likelihood of the fish being eaten by the parasite's definitive host. In our study, however, this was not specifically tested, and as such conclusions about the adaptiveness of the behavioral change should be made with caution. We have acknowledged various factors which could have influenced our findings and suggest that future studies endeavor to test them specifically with respect to *T. darbyi* infection. One way to account for several factors (e.g., innate active behavior) would be to experimentally infect bullies taken from a population lacking the parasite. Unfortunately, despite screening hundreds of snails, we have still not identified the snail first intermediate host of *T. darbyi*, and as such obtaining the larval stage infective to common bullies is not yet possible. When the snail host is found, conducting experimental infections will allow characterization of behaviors pre- and post-infection as well as robust within-population comparisons of infected and uninfected individuals.

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## Compliance with ethical standards

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the University of Otago Animal Ethics Committee (# 70/17001).

**Conflict of interest** The authors declare that they have no conflict of interest.

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