How might *Gyrodactylus* parasitism modify trade-offs between female preference and susceptibility of males to predation in Trinidadian guppies?

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A B S T R A C T

A number of examples exist of trade-offs between mating success and survival; that is, success in one fitness component comes at the cost of success in the other fitness component. However, these expected trade-offs are – perhaps even more commonly – not observed. One explanation for this apparent paradox of missing trade-offs could be that the other factors generating fitness variation across individuals confound or obscure the expected trade-off. These confounding effects could arise in two general ways: (i) the additional source of variation could positively (or negatively) influence both fitness components ("shared confounder" hypothesis), or (ii) the additional source of variation could influence only one fitness component ("non-shared confounder" hypothesis). We tested whether parasitism by *Gyrodactylus* spp. could be a confounder of trade-offs between female preference and susceptibility to predation for male Trinidadian guppies (*Poecilia reticulata*). As in previous work, we did not find the expected trade-off; that is, the males preferred by females were not more likely to be eaten by predators. Because half of the experimental males were infected by *Gyrodactylus* in a paired design, we were able to show that females discriminated against infected males, but that infected males were not more susceptible to predation. Our results thus provide support for the non-shared confounder hypothesis. That is, by negatively affecting one fitness component (female choice) but not the other (susceptibility to predation), parasitism by *Gyrodactylus* could obscure the expected trade-off between female preference and susceptibility to predation.

1. Introduction

Individuals with conspicuous secondary sexual traits are expected to attract more mates at the expense of increased susceptibility to predators (e.g., Endler, 1980; Rosenthal et al., 2001). This expected "trade-off" between natural and sexual selection has been examined for many traits, including colour (e.g., Endler, 1980; Johnson and Candolin, 2017), acoustic signaling (e.g., Kotiaho et al., 1998), and physical ornaments (e.g., Hernandez-Jimenez and Rios-Cardenas, 2012). However, despite some confirmatory examples, trade-offs are not always observed (Metcalf, 2016), including in our own work (e.g., de Lira et al., 2018). Further, a meta-analysis across taxa, traits, and studies found that males with more conspicuous traits (e.g., larger ornaments or increased courtship rates) actually had higher average survival (Jennions et al., 2001). Because the fitness components (e.g., survival, mating success, fecundity) that underlie trade-offs can be affected by

many sources of variation, investigating the interactive effects of those sources might provide insight into this apparent paradox of missing trade-offs.

In natural environments, many sources of selection can generate fitness variation across individuals (Endler, 1986; Wade and Kalisz, 1990; MacColl, 2011), including mate choice (e.g., Andersson and Simmons, 2006), predation (e.g., Genovart et al., 2010), parasites (e.g., Cable and van Oosterhout, 2007), competition (e.g., Calsbeek and Cox, 2010), and pollution (e.g., Bashalkhanov et al., 2013). Each of these sources of variation can influence the same or different fitness components, and thus can alter the observable effects of any one source of selection (Møller, 2008). One example is seen in the signaling behaviours of lesser wax moths (*Achroia grisella*). Male lesser wax moths produce sexual signals that are known to attract mates, but that also attract bat predators (Edomwande and Barbosa, 2020), thereby establishing a possible trade-off between mating and survival. Yet, when signaling in groups, and especially in groups with attractive competitors (competition for mates is high), males have been found to produce riskier signals with fewer and shorter silent

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periods (Edomwande and Barbosa, 2020). In this case, possibly due to decreased predation risks and increased competition for mates (Edomwande and Barbosa, 2020), competition appears to alter the expected costs of sexual signaling. As another example, parasitism can modify many ecological interactions, including predator–prey dynamics (e.g., Hudson et al., 1992; Joly and Messier, 2004), mate choice (Deaton, 2009), intra– and inter–specific competition (Price et al., 1986), and even mutualism (Gillespie and Adler, 2013). These complex interactions, where one source of selection can influence the interactions between other sources of selection, present important challenges to understanding trade-offs in natural contexts.

An expected trade-off between two fitness components can be obscured by a third source of variation in at least two general ways. First, the additional factor could have similarly positive or negative effects on both fitness components that would otherwise trade-off with each other. For example, individuals with high energy levels can afford greater investment (relative to individuals with low energy) in both mating and survival, thus eliminating the trade-off that would be expected if all individuals had similar energy levels (van Noordwijk and de Jong, 1986; de Jong and van Noordwijk, 1992). As another example, if some individuals are infected by parasites, then infection could reduce the success of those individuals (relative to uninfected individuals) in both mating and survival. Second, the additional factor could introduce variation into one fitness component but not the other. For example, if parasitism influences mating success but not predation risk, and if some individuals are infected by parasites, then the "noise" that parasitism adds to the system will obscure the trade-off between mating success and susceptibility to predation. For simplicity, we henceforth refer to the first possibility as the "shared confounder" hypothesis and the second possibility as the "non-shared confounder" hypothesis.

We here investigate the effects of parasitism on female preference (female mate choice) and susceptibility to predation (predator avoidance) of males, as well as the expected trade-off between these two fitness components. Past studies have shown that parasitism can influence each of these fitness components in some instances; for example, females of some species discriminate against infected males (e.g., Clayton, 1990; Zuk et al., 1990; Ehman and Scott, 2002) and parasite infection in some species can influence their vulnerability to predators (e.g., Hudson et al., 1992; Joly and Messier, 2004). However, we are not aware of any studies that have asked how parasitism influences female preference for – and predation on – the same individuals. By adopting this last approach, we hope to gain insight into how one source of variation (parasitism) might influence interactions between two other fitness components (female preference and susceptibility to predation), and whether the outcome is consistent with the shared confounder or the non-shared confounder hypotheses.

Trinidadian guppies (Poecilia reticulata) are an excellent system to investigate these interactions because effects of parasitism on female preference and susceptibility to predation are well-known, and because trade-offs between natural and sexual selection are well documented (although those have not always been found). For example, male guppies will sometimes attempt to court females by performing sigmoidal displays but, when predation risks are high, males display less often (Godin, 1995). As another example, females often prefer to mate with more colourful males (Houde, 1987), yet bright colour can leave males more susceptible to predators (Endler, 1983; Godin and McDonough, 2003; Millar et al., 2006; Weese et al., 2010). A particularly important predator is the pike cichlid (Crenicichla sp.; Reznick and Endler, 1982), which is a visual predator that commonly preys on guppies in nature (Reznick and Endler, 1982; Seghers, 1973). Analysis of geographic variation in the antipredator adaptations of the guppy: Poecilia

reticulata. Doctoral dissertation, University of British Columbia, Canada). Crenicichla predation is considered to be a primary driver for the divergence of colour among guppy populations, as males tend to be less bright and have fewer colour patches where Crenicichla are present (Endler, 1980). Male guppies thus seem to experience a trade-off, where males that are more successful in obtaining mates also suffer from increased predation. How might such interactions – and trade-offs – be influenced by parasitism?

In their native range in Trinidad, guppies typically coexist with Gyrodactylus spp. (referred to as Gyrodactylus only hereafter), monogenean ectoparasites that can have important implications for fish fitness (Fig. 1), including in relation to mate choice and susceptibility to predation (Bakke et al., 2007). The pathology associated with Gyrodactylus infections often involves increased feeding rates (Kolluru et al., 2009), lethargy (van Oosterhout et al., 2003), erratic behaviours (Cable et al., 2002), and increased mortality (van Oosterhout et al., 2003; Cable and van Oosterhout, 2007). Infected males, in particular, reallocate resources to immune functioning at the expense of sexually selected traits such as courtship behaviours (Kolluru et al., 2009) and carotenoid-based colours (Houde, 1987; Kodric-Brown, 1989; Houde and Torio, 1992). Considering these effects, Gyrodactylus parasitism could have important effects on both female preference and susceptibility of males to predation, and thus the potential trade-off between these two fitness components. Yet the effects of parasitism on female preference and susceptibility to predation have not been assessed for the same individual males – which is the critical level for examining trade-offs.

In our previous work (de Lira et al., 2018), we tested for the trade-off between female preference and susceptibility to predation. That test was performed at the level of the whole organism (i.e., are the males preferred by females also more likely to be killed by a predator), rather than at the level of individual traits (e.g., colour or behaviour or size or shape), because many traits integrated at the whole organism level will influence both fitness components (see de Lira et al., 2018). Extending this earlier work, we here add variation in an additional factor (Gyrodactylus parasitism) by conducting the experiment with pairs of males: one infected and one uninfected. With this new design, we were able to ask whether parasitism might influence the potential trade-off between female preference and susceptibility to predation – in either of the two general ways introduced above. For the shared confounder hypothesis, parasitism could cause infected males to perform worse with mates and for those same males to be more susceptible to predation. For the non-shared confounder hypothesis, parasitism could influence only one of those two factors.

2. Materials and methods

2.1. Fish collection, and housing

We collected male and female guppies in the lower Arima River in Trinidad, a high-predation locality where guppies co-exist with Crenicichla. We immediately transported the captured guppies to the nearby field laboratory (William Beebe Tropical Research Station) in individual 237 mL bags (Whirl-Pak, USA) to prevent any potential movement of Gyrodactylus between guppies. To further ensure that transmission did not occur in captivity, guppies were individually housed in the laboratory. These isolation procedures ensured that the parasite load on an individual fish was an accurate representation of a fish's infection status in the wild.

Once in the laboratory, guppies of both sexes were individually anesthetized in 0.02% Tricaine Methanesulfonate (MS-222) (1:8000) (Finquel, Argent Labs, USA) buffered to a neutral pH using NaHCO3. Given that all males – whether infected or not – were
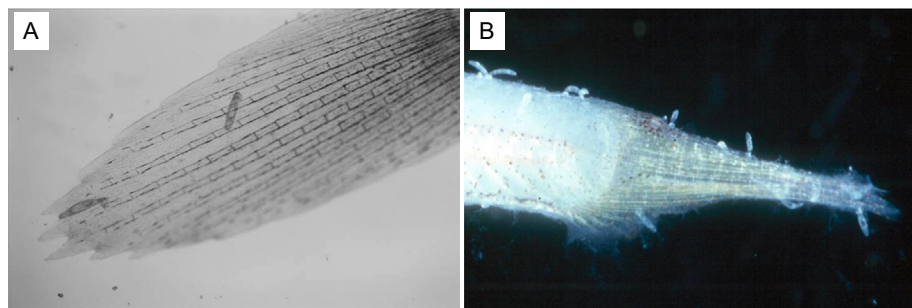
Fig. 1. Guppies infected with *Gyrodactylus*, showing (A) a couple of *Gyrodactylus* on a guppy fin with little visible damage, and (B) several *Gyrodactylus* infecting a highly damaged guppy. Images courtesy of Dr Marilyn E. Scott, McGill University, Canada.

exposed to MS-222 and then allowed to recover for at least 1 day, we do not think that the procedure would have persistent effects on colour, female preference, or predator avoidance. Further, short recovery times (30 min) after exposure to MS-222 have been reported for other fish species (Nordgreen et al., 2014). While anaesthetized, the guppies were checked for parasites by fully scanning the body surface using a dissecting microscope (Zeiss, Canada) under illumination by a cold light source. Using this standard procedure (e.g., Pérez-Jvostov et al., 2012; Gotanda et al., 2013a), individuals could be sorted according to parasite status and sex. A total of 36 uninfected females in good observable health were selected, as were 72 males (36 infected and 36 uninfected). Note that the guppies used in our experiment were either naturally infected or uninfected; that is, we did not do any experimental infections. To ensure that the uninfected males were truly uninfected before being used in the experiment, the uninfected males were treated with an aqueous solution of Clout (Sentry AQ) (Fritz Aquatics, USA), which eliminates all parasites, including any potential *Gyrodactylus* that we might have missed. Although we are not aware of any documented side effects of Clout, males were never used on the same day that they were treated, and they all appeared to be fully recovered during the day of the trial. Females were not treated with Clout.

We also collected three *Crenicichla* – one from each of three distinct rivers (Aripo, Quare, and El Cedro) and held them individually in 150 L containers, each with a clay shelter. These containers were placed in a shaded area outdoors at the William Beebe Tropical Research Station. All three *Crenicichla* were fed wild guppies (both males and females) from the Arima River once a day for multiple weeks prior to the experiment. Given that the *Crenicichla* were successfully consuming the guppies that were placed in their tanks, and as all *Crenicichla* were adults that were captured in the wild, these individuals were effective visual predators and proficient at catching guppies.

Among the guppies that we collected, the mean intensity of *Gyrodactylus* was two (the mean number of *Gyrodactylus* per infected individual), with a minimum of one, and a maximum of six. For the same population (the lower Arima), Gotanda et al. (2013b) found that guppies had an average of two parasites (note that their estimate includes uninfected individuals), and prevalence was 53% (the proportion of individuals in the population that were infected with *Gyrodactylus*). Gotanda et al. (2013b) reported even lower numbers in the localities where we collected predators, documenting an average of less than one *Gyrodactylus* per individual for all three sites.

2.1.1. Trial preparation

The males selected for each trial (one infected and one uninfected) were approximately size matched to control for possible effects on parasitism, female preference, and susceptibility to predation (Karino and Matsunaga, 2002; Johansson et al., 2004; Cable and van Oosterhout, 2007). Linear mixed effects models (LMMs) with body size as the response variables, infection status (i.e., infected or uninfected) as the explanatory variable, and trial as a random effect, confirmed the effectiveness of this size-matching in that the two males paired in a trial did not differ in body mass (LMM – Estimate(Infection status (Uninfected)) = −0.00, 95% confidence interval (CI) = −0.002 to 0.00, P = 0.227) or body area (LMM – Estimate(Infection status(Uninfected)) = −3.05, 95% CI = −8.23 to 2.11, P = 0.25). Males were not colour-matched as this would be logistically impossible in the field while also trying to match body size and to select infection status. However, an LMM with colour (Principal component (PC) 2 – see Section 3) as a response variable, infection status as an explanatory variable, and trial as a random effect, showed that there were no average colour differences between the infected or uninfected males (LMM – Estimate(Infection status(Uninfected)) = −0.445, 95% CI = −1.09 to 0.203, P = 0.182). Of course, they did differ in colour within each trial, which had the benefit of allowing us to individually identify males without having to artificially mark them.

One female and a pair of males (one infected and one uninfected, matched by size) were selected for each experimental trial. Once males were assigned as a pair, they remained together for both the female preference and predator trials. On the day prior to the trial, the guppies were re-anaesthetized to confirm their infection status (infected or not). Males were then photographed with a Nikon D7000 DSLR with a 60 mm macro-lens under full-spectrum lights following the methods of Gotanda et al. (2013a). These photographs were used to quantify some aspects of male colour. All individuals were then housed separately overnight in recovery aquaria.

2.1.2. Female preference trial

Trials were conducted between 08:00 h and 15:00 h in 51 cm length × 26 cm width × 31 cm height aquaria, filled with water until close to full, with shaded walls to limit visual disturbance. These aquaria were divided into three sections using glass dividers, with each of the two side compartments (on either side of the central compartment) measuring 16 cm long. A black screen along the base of each divider allowed water flow between the sections, which is probably one way that females detect a male's infection status (Stephenson et al., 2018).

To assess female preference for infected versus uninfected males, we used a modified dichotomous choice test. Tests of this type are commonly used to assess preference in guppies (e.g., Evans et al., 2004; Auld et al., 2016; Kniel and Godin, 2020), where preference is assigned to the one of two individuals (in this experiment, one of the two males in each pair) with which the focal individual (in this experiment, a single female) spends more time. In each trial, the infected male and the uninfected male were assigned to the peripheral (at either end) sections of the aquarium, and the female was placed in the middle. The sides that the males

were assigned to were randomly selected to help account for possible biases in side preference (e.g., Gatto et al., 2017). Prior to the start of the trial, individuals were given a 20 min acclimation period, during which time opaque placards prevented the fish from seeing each other. Once the placards were removed, an additional 5 min were given to ensure the female noticed both males. The time that both a male and the female were within 4 cm of the same glass partition, and where the female was oriented towards that male, was recorded until a cumulative total of 5 min had been spent between the males.

2.1.3. Predator preference trial

After the above female preference trials, the same pairs of infected and uninfected males that were together in the preference experiment were transferred with a small net into a tank with a single predator. As the prior experience of the predator could affect their preference or ability to catch the guppies, the specific predator tank was randomly selected from among the three individual predators. Additionally, the *Crenicichla* were not fed on the day of the trial until after the trial was complete, and there was only one trial per day per predator. Using a hand-net, the guppies were introduced into the tank at the same time, and they were left with the predator for 60 min, or until one of the males was eaten. The surviving male was then removed, anaesthetized using MS-222, and identified using the photographs taken during initial processing. In six cases, neither male was eaten; and, in three cases, both males were consumed before the observer was able to remove the surviving male from the tank. These instances were included in the female preference analyses but were excluded from the predation and multifactorial analyses.

2.1.4. Male colour

Colour is only one of many factors that can influence female preference, susceptibility to predation, and parasitism. As some aspects of colour could be easily measured from the photographs, we considered how these aspects might influence the effects of parasitism – and its interaction with female preference and susceptibility to predation. Colour was measured for each male following the methods of Gotanda et al. (2013a). Using ImageJ (Schneider et al., 2012), we measured the body area, the number of individual colour spots, their relative areas, and the total colour area (body area/sum of all colour spots area). We then classified each colour spot as black, fuzzy black, orange, yellow, violet, silver, and green. Many other aspects of colour can influence interactions between male guppies and female preference, susceptibility to predation, and parasitism. However, the aspects we measured are similar to a large set of studies considering colour variation in guppies. Importantly, our focus was not specifically on colour (rather it was on interactions between female preference, susceptibility to predation, and parasitism) – and we here include colour only to consider its potential moderating influence on those interactions.

2.1.5. Statistical analysis

To test for a trade-off between female preference and susceptibility to predation while ignoring parasitism, we first investigated whether females preferred (spent more time with) the male that was later eaten by the predator (relative to the male that was not eaten) in a two-tailed one-sample t-test against the expectation if females showed no preference (i.e., 0.5). The response variable was the proportion of time that females spent with the male that was later eaten (seconds with eaten male/total trial time). Note that this approach does not imply a test for whether female choice has a causal influence on a male's later consumption by a predator. Rather, it seeks to uncover the possibility that some underlying causal factor, such as a trade-off, influences both female preference and susceptibility to predation.

We next investigated the effects of parasitism individually on each fitness component: female preference and susceptibility to predation. For susceptibility to predation, we conducted a one-sample Chi-Squared test with the infected males – thus asking whether those males were equally likely to be eaten by a predator (eaten – yes/no) – relative to the paired uninfected male. For female preference, we conducted tests of two types. The first test exactly mimicked the structure of the predator test described above, thus allowing direct comparison of effect sizes of parasitism on the two fitness components. In this case, we conducted a one-sample Chi-Squared test with the infected males – thus asking whether those males were equally likely to be preferred by a female (preferred – yes/no) – relative to the paired uninfected male. The second test leveraged quantitative variation in female preference: i.e., the proportion of time that a female spent with the uninfected male in a trial (seconds with uninfected male/total trial time). Here we used a two-tailed one-sample t-test against the expectation if females showed no preference (i.e., 0.5). This second test mirrors the classic approach used in many studies of dichotomous mate choice trials (e.g., Evans et al., 2004; Auld et al., 2016; Kniel and Godin, 2020).

We next considered all three factors (female preference, susceptibility to predation, parasitism) together in generalized linear models (GLMs) to ask how inclusion of information about parasitism might influence the trade-off between female preference and susceptibility to predation. We used the "lme4" package (Bates et al., 2015) for all modelling, and all analyses were done using R version 4.0.2 (R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). The chosen response variable for the GLMs was the one most amenable to analysis in the GLM: the proportion of the total time that a female spent with the preferred male (i.e., the male with which she spent more time). Because this variable was a proportion, all GLMs were modelled with binomial distributions. The first model considered only whether the male was eaten or not and thus directly paralleled our initial t-test (above) for a trade-off in the absence of information about parasitism (i.e., Proportion of time with the preferred male ~ Eaten by a predator). In the second model, we added information about parasitism (the number of parasites: among all the males preferred by females, min = 0, mean = 0.65, max = 5; among only the infected males preferred by females, mean = 1.5). In doing so, we asked how adding parasitism would influence evidence of a trade-off between female preference and susceptibility to predation (i.e., Proportion of time with the preferred male ~ Eaten by a predator * Number of parasites). Examining the nature of the interaction between female preference and susceptibility to predation would then help us to distinguish, at least qualitatively, the two hypotheses (shared confounder or non-shared confounder) through which a third factor (parasitism) might influence the trade-off between two other factors (female preference and susceptibility to predation). Note that although *Gyrodactylus* intensity is low, low intensities are common in natural populations (see Section 4). Further, although the particular predator could have influenced the outcome of each trial, we were unable to include predator identity as random effects in our GLMs, because inclusion of predator identity would result in our models being singular fits.

Finally, we added male colour to the final GLM to see if information about this one class of male traits might alter any of the conclusions. To reduce dimensionality of colour variables, we first conducted a Principal Component Analysis (PCA) on all male colour variables using the "*factoextra*" package (Kassambara and Mundt, 2020. Factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7: 2020). Aspects of green area contributed the most (30.36%) to PC1, which accounted for 18.1% of the total variance explained. Total colour area (28.59%) and aspects

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of orange area (19.99%) contributed the most to PC2, which accounted for 17.4% of the total variance. PC2 was selected for addition to the final GLM as total colour area and orange area have been found to be important for female preference (Endler and Houde, 1995) and susceptibility to predation (Weese et al., 2010; Gordon et al., 2015). The structure of this final GLM was thus: Proportion of time with the preferred male $\sim$ Eaten by a predator * Number of parasites * PC2. Correlations between our explanatory variables were assessed with phi-correlations (for infection status and susceptibility to predation) and point biserial correlations (for infection status/susceptibility to predation and PC2), and we found that none were correlated.

2.1.6. Data accessibility

The raw data for this experiment are available at MendeleyData (https://doi.org/10.17632/hdwzmvrc94.1).

2.1.7. Ethical statement

To understand the effects of parasitism on susceptibility to predation, we chose to directly measure predation of the guppies. This decision was necessary to understand the effects of parasitism on survival with a predator. Following ethical recommendations (e.g., Huntingford, 1984), we limited the number of subjects used in this experiment. Once the experiments were complete, the guppies that were not eaten were returned to their natural populations. The work in this study was approved under the Animal Use Protocol AUP 2012-7257.

3. Results

Considering only female preference and susceptibility to predation in a simple t-test, our data did not reveal evidence of a trade-off. That is, females spent the same amount of time with males that were eventually eaten by predators and those that were not (Fig. 2; $t_{22} = -0.93$, lower 95% confidence interval (LCI) = 0.35, upper 95% confidence interval (UCI) = 0.56, $P = 0.36$). We then considered how parasitism might influence each fitness component individually. First, we found no evidence that parasitism influenced predation on males (in 52% of the trials the parasitized male was eaten:

$X^2 = 0.04$, degrees of freedom (df) = 1, $P = 0.83$). Second, in the parallel test of female preference, the effect size of parasitism was much higher (in 38% of trials the parasitized male was preferred) although significance was lacking ($X^2 = 2$, df = 1, $P = 0.16$). Using the more powerful test leveraged quantitative information on female preference (proportion of time with preferred male); however, our results may suggest (although significance was lacking) that females preferred uninfected males over infected males (Fig. 3; $t_{31} = 1.76$, LCI = 0.49, UCI = 0.67, $P = 0.09$).

We then used GLMs to consider all three factors together (female preference, susceptibility to predation, and parasitism). The first GLM paralleled our initial t-test above: that is, it tested for a trade-off between female preference and susceptibility to predation in the absence of information about parasitism. Not surprisingly, it yielded the same outcome: males that were preferred by females were not more likely to be eaten by a predator (Estimate: Not eaten = 0.04, $P = 0.96$). The next GLM added parasitism as an additional predictor variable. Nothing was significant in this model (Table 1). However, visualizing the interaction (Fig. 4) suggested a trend consistent with the idea that parasites might obscure the trade-off between female preference and susceptibility to predation. That is, the trade-off appears possible (the trend is in the right direction) for uninfected males (i.e., those favoured by females seem more likely to be killed by predators), but no trend is evident for infected males. This change (again ignoring significance) is consistent with the non-shared confounder hypothesis where parasitism introduces variation into one fitness component (female preference) but not the other (susceptibility to predation). Adding male colour variables to this last GLM did not affect the proportion of time that females spent with a preferred male, either on its own, or in an interaction with predation (eaten or not eaten), or parasitism (infected or uninfected) (Supplementary Table S1).

4. Discussion

We investigated whether *Gyrodactylus* parasitism could influence the expected trade-off between female preference and susceptibility to predation in male Trinidadian guppies. We then considered whether our results were more consistent with the

Fig. 2. Female preference in each trial for either the male that was eventually consumed by the predator, or for the male that survived. Each of the jittered data points represent a trial ($n = 23$), where the time spent with the eaten male was divided by the total trial time (300 s). Circles represent trials where a female spent more time with the male that was later eaten, whereas triangles are the trials where females spent more time with uneaten males. The horizontal dashed line indicates the proportion of time where there would be no preference (above the horizontal line shows preference for eaten, below shows preference for uneaten). The large point represents the sample mean, and the error bar represents the lower 95% confidence interval obtained from the one-sample t-test (there is no upper limit to the confidence interval, as the t-test was one-tailed).
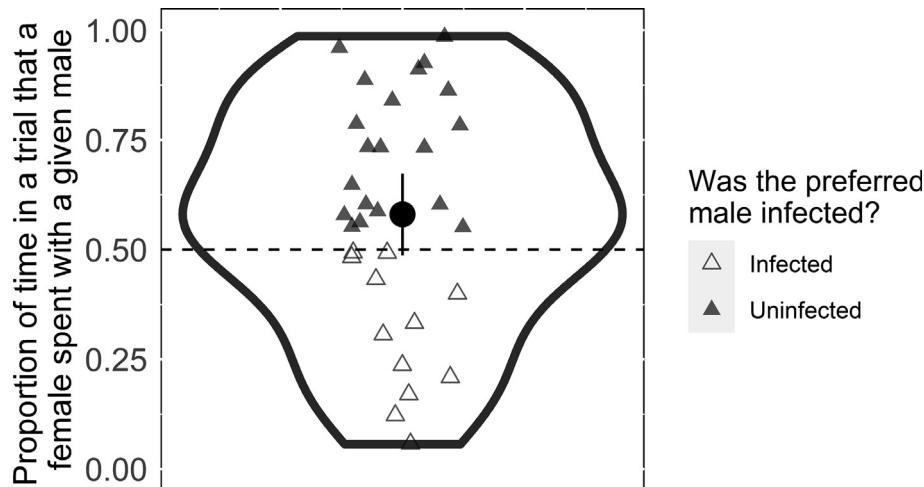
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Fig. 3. Female guppy (*Poecilia reticulata*) preference in each trial for either the infected or uninfected male. Each of the jittered data points represent a trial (*n* = 32), where the time spent with uninfected males in that trial was divided by the total trial time (300 s). Triangles represent trials where a female spent more time with the uninfected male, whereas circles are trials where females spent more time with infected males. The horizontal dashed line indicates the proportion of time where there would be no preference (above the horizontal line shows that the female preferred the uninfected male, below shows that the female preferred the infected male). The large circular point represents the sample mean, and the error bar represents the lower 95% confidence interval obtained from the one-sample t-test (there is no upper limit to the confidence interval, as the t-test was one-tailed).

Table 1
Generalized linear model results for the effect of parasitism (number of parasites: min = 0, max = 5, mean = 0.65), predation (eaten by predator: eaten or not eaten) and their interaction on the proportion of time spent by a female with the preferred male in a trial.

Predictors	Odds Ratios	Proportion of time with the preferred male	
		95% CI	P
(Intercept)	2.99	0.51–31.31	0.264
Eaten by predator [Not eaten]	0.91	0.07–8.91	0.934
Number of Parasites	0.63	0.06–5.63	0.652
Eaten by predator [Not eaten] * Number of Parasites	1.34	0.13–14.85	0.795
Observations		23	

CI, confidence interval.
The asterisk indicates an interaction between the two variables.

"shared confounder" hypothesis (parasitism has negative effects on both fitness components) or the "non-shared confounder" hypothesis (parasitism introduces variation into only one fitness component). As in our previous work (de Lira et al., 2018), we did not detect a trade-off because the males that were preferred by females were not more likely to be eaten by a predator. Because half of the males were infected in a paired design, we were then able to demonstrate that females discriminated against infected males but that infection status did not matter for susceptibility to predation. Finally, by adding parasitism to the models testing for a trade-off between female preference and susceptibility to predation, we were able to suggest (without statistical significance) that the "non-shared confounder" effect might obscure the expected trade-off. Below, we discuss effects of parasitism on the individual components and their potential trade-off, before providing suggestions for future research.

We start by considering our finding that parasitism of males negatively influenced female preference. Several factors could explain this outcome. First, females might be able to sense if a male is infected and then avoid that male to prevent direct transmission or due to an "expectation" of his low genetic quality (Houde and Torio, 1992; Stephenson et al., 2018). Second, the energetic costs of infection could diminish the effectiveness of male displays (e.g., colour or courtship behaviour) which then could negatively influence female preference. With respect to colour, some laboratory studies of guppies have reported differences between infected and uninfected males (Houde and Torio, 1992); yet such an associ-

ation has not been detectable in nature (Martin and Johnsen, 2007; Gotanda et al., 2013a). In our study, colour did not differ between infected and uninfected males within a trial (see Section 2.1.1) and including information about male colour did not alter any of our general conclusions (Supplementary Table S1). With respect to courtship behaviour, infection can reduce male display rates, although the effects of infection on male attractiveness to females are mixed (e.g., Kennedy et al., 1987; Houde and Torio, 1992; López, 1998; Kolluru et al., 2009). Logistical constraints prevented us from measuring male courtship behaviour, but these and other variables (e.g., other aspects of colour and other parasites) could be considered in future work.

It is noteworthy that the females in our study discriminated against males infected with an average of only two *Gyrodactylus*, suggesting that even relatively low infection loads can be important for this fitness component. One explanation for how females were able to discriminate against males with so few parasites could be that the males had been infected for long periods of time prior to the experiment. Indeed, although the infection duration and prior infection status of the males in our study are unknown (because they were collected from nature), the low intensities that we observed are not inconsistent with longer infection durations. That is, when *Gyrodactylus* initially infect a host, they grow exponentially in the short-term before declining to a low intensity or elimination (Richards and Chubb, 1998). Hence, guppies with only two current parasites might well have experienced the effects of recently higher infection loads – and these earlier
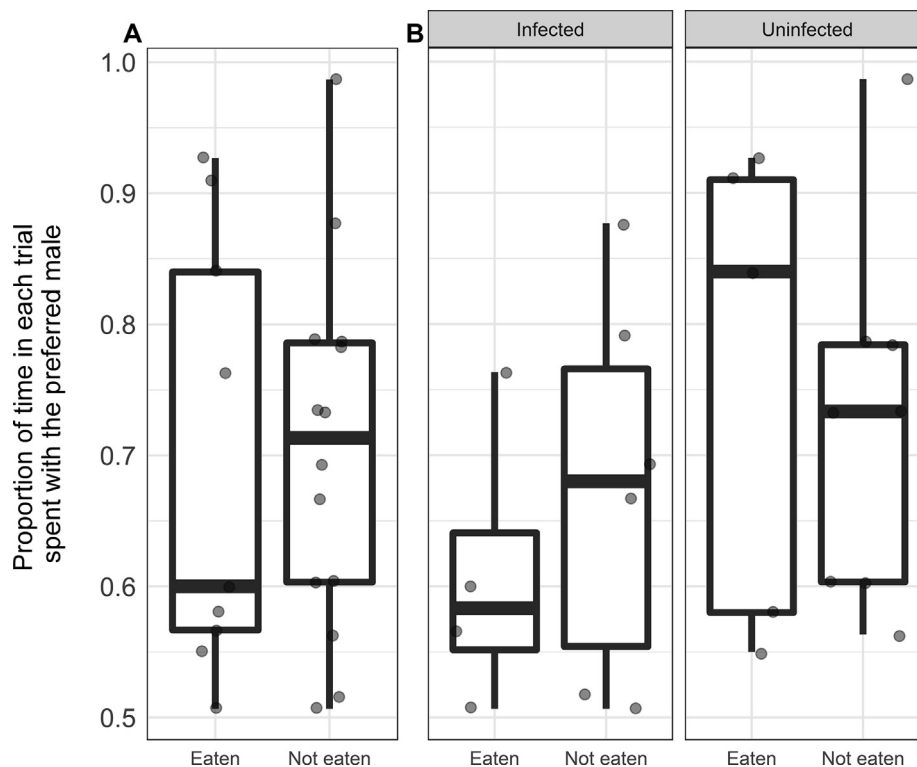
Fig. 4. The relationship between female preference and predation of the preferred males in a trial, (A) overall, and (B) faceted by infection status. Each jittered data point represents a trial ($n = 23$), the time spent with the preferred male was divided by the total trial time (300 s).

effects might have influenced male displays or other cues detectable by females. We consider the possibility of side effects from Clout to be a necessary limitation of this study (although we are not aware of any side effects). Working with naturally infected males is advantageous for understanding parasite infections as they occur in nature, and this would not have been possible to the same extent, without the use of a de-wormer like Clout. Although this treatment may potentially have had an effect on female preference, we are unaware of any formal studies where this has been tested.

We now consider our finding that parasitism of males did not influence susceptibility to predation. Several potential explanations for this result are biological (as opposed to methodological). First, the infection intensities could have been too low to compromise predator detection, avoidance, or escape. This possibility aligns with suggestions made by other researchers that low infection loads might not have dramatic effects on guppy survival in the wild (van Oosterhout et al., 2003). Second, our experiment considered only one type of predator and one type of parasite, whereas such associations could vary depending on which parasites and predators are involved. Third, the guppies in our experiment could be those that were the best at escaping from predators. That is, the males used for our experiment had been successful in surviving to adulthood under high predation risks in nature even when infected by parasites. Hence, they might have been – in essence – preselected as those most capable of avoiding predation even while infected. Studies of wild-caught individuals are always subject to such unknown effects of prior experience or selection within a generation, the latter sometimes called the "invisible fraction" (Grafen, 1988).

Other explanations for parasitism not influencing susceptibility to predation in our study could be related to our study design and experimental apparatus. For instance, as discussed above, there could be subtle side effects of the parasite treatment that was used

on half the males. Further, our use of pairs of males precluded the formation of larger shoals. Shoaling behaviour is known to be an important anti-predator defense in some populations (Seghers, 1974; Song et al., 2011), and this behaviour can be impacted by parasitism (Hockley et al., 2014). We also did not allow the guppies time to acclimate after introduction to the tank with the predator. Although both guppies were introduced to the tank in the same way at the same time, it is possible that they were disoriented and thus could not mount appropriate predator avoidance. And, of course, the particular design of our predation arenas could have dictated the nature of predator–prey interactions, as is the case in all experimental studies. Further work with other study designs and experimental arenas will be necessary to evaluate these and other possibilities.

We now return to the apparent lack of a trade-off between female preference and susceptibility to predation. Our finding here was consistent with a series of laboratory experiments that we previously conducted with guppies that were all treated to eliminate parasites (de Lira et al., 2018); and with other researchers who have failed to demonstrate such trade-offs in a variety of taxa (see Section 1). van Noordwijk and de Jong (1986) provided what we here call the "shared confounder" hypothesis for such missing trade-offs, theorizing that individuals differ dramatically in their total resources. In our case, this explanation could apply if parasitism of males negatively influenced both female preference and survival when faced with a predator. An alternative explanation, which we here call the "non-shared confounder" hypothesis is that an external factor introduces variation into only one component of the trade-off. Our results provide support consistent with this second hypothesis: that is, parasitism introduces variability into female preference but not susceptibility to predation. Adding information on parasitism into the trade-off model yielded estimates consistent with this hypothesis – although statistical significance was lacking.

We now consider how natural variation in the guppy system might be leveraged in future work. In particular, female preference, susceptibility to predation, and parasitism vary dramatically across populations of guppies in Trinidad. Female preference, for example, varies among populations in relation to a number of colour, size, shape, and behavioural characteristics of the males (Endler and Houde, 1995), as well as due to variation in the external environment (e.g., Gamble et al., 2003). As such, females in some populations can be indifferent towards, or show a dislike for, male traits that are preferred in other populations (Endler and Houde, 1995; Brooks and Endler, 2001). Similarly, predation varies dramatically among populations (Endler, 1978; Reznick et al., 1996; Weese et al., 2010). Some of this variation is associated with predation "regime" – as in the classic low-predation versus high-predation contrast (Endler, 1978; Reznick et al., 1996). Even within a predation regime, however, predators can vary dramatically – especially between the north and south slopes, which have different predator faunas (Reznick et al., 1996). Further, the abundance of a given predator can vary dramatically among tributaries and among pools within tributaries even for a particular predator regime on a given slope (Endler, 1978; Millar et al., 2006). Finally, *Gyrodactylus* abundance and prevalence is highly variable across guppy populations in Trinidad (Gotanda et al., 2013a; Stephenson et al., 2015). It is reasonable to assume that a past history of exposure to parasites will influence the response of guppies from a given population to infection – and, indeed, such effects have been documented (Dargent et al., 2013; Pérez-Jvostov et al., 2015).

We suggest that this complex matrix of among-population variation in female preference, susceptibility to predation, and parasitism could be leveraged to explore the evolution of trade-offs and their sensitivity to parasitic infection. For example, we would expect females to be better at avoiding infected males in populations where infection is common but not universal – a situation in which selection should favour strong discrimination. Further, we would expect parasitism to have a stronger effect on predation in populations where parasitism is lower – because then guppies would be under lower selection for tolerance to infection. Finally, particular combinations of female preference, susceptibility to predation, and parasitism might generate different trade-offs and confounders – although it is difficult to predict the specifics in advance. We further recommend studies that alter the experimental procedures (examples were noted above), that use controlled experimental infections, that use wild-caught and laboratory-raised individuals, and that examine additional potential confounders (e.g., energy stores, other parasites, different predators). The paradox of missing trade-offs is persistent in evolutionary biology (Jennions et al., 2001; Metcalf, 2016; de Lira et al., 2018) and the exploration of potential confounders is a potential solution to this paradox.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2022.01.006.

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