

A field test of the Hamilton–Zuk hypothesis in the Trinidadian guppy (*Poecilia reticulata*)

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Abstract The Hamilton–Zuk hypothesis proposes that females prefer male secondary sexual traits because they are honest indicators of parasite resistance. Despite the attention that this hypothesis has received, its role in sexual selection remains equivocal. This study presents the first field test in guppies of two key predictions of the Hamilton–Zuk hypothesis: (1) that within populations, the most highly ornamented males have the fewest parasites and (2) that among populations, males in high parasite populations have the most conspicuous ornaments. Five hundred male guppies from 19 distinct populations in the Northern Range of Trinidad were inspected for *Gyrodactylus* parasites and photographed. Eight measures of orange spot ornamentation were used to test the predictions: hue, saturation, lightness, relative area, number, and area-weighted hue, saturation, and lightness. Parasite load had no significant effect on any of these measures. There was also no relationship between orange spot ornamentation and parasite abundance among populations. Guppies from high-predation environments had significantly more parasites, and their orange coloration was lighter and less saturated than that in guppies from low-predation environments. Despite previous lab results, this study found no relationship between parasite load and male orange spot ornamentation.

Keywords Female choice · “Good genes” models · Guppy · *Gyrodactylus* · Hamilton–Zuk hypothesis · *Poecilia reticulata* · Sexual selection

Introduction

Sexual selection by female mate choice was first conceived by Darwin (1871) as an explanation for elaborate, seemingly maladaptive male traits. However, debate still surrounds the origin of female preferences in species where females receive no direct benefits from males, specifically whether preferences are initially random (Fisher 1930), sensory-biased (Basolo 1990; Ryan 1990), or provide genetic benefits (Trivers 1972). “Good genes” models propose that females prefer certain male traits because they are honest indicators of high fitness alleles that will enhance the fitness of their offspring (Trivers 1972). However, “good genes” should quickly become fixed in a population under this selection regime, reducing heritable variation in fitness and, consequently, relaxing sexual selection (Maynard Smith 1978). The hypothesis of Hamilton–Zuk (1982) resolves this issue by proposing that certain male traits are correlated with parasite resistance. Parasite–host coadaptational cycles result in continuous fitness variation. In this context, male traits may provide a stable beacon guiding females to males resistant to the current parasite strains. Despite the substantial interest generated by Hamilton and Zuk’s hypothesis, no consensus has yet been reached on its validity (reviewed by Møller 1990; Read 1990; McLennan and Brooks 1991; Hamilton and Poulin 1997; Møller et al. 1999).

The guppy *Poecilia reticulata* is an ideal system in which to test the Hamilton–Zuk hypothesis due to extensive work on natural and sexual selection in this species (reviewed by Endler 1995; Houde 1997). This small poeciliid fish is native to freshwater rivers and streams on the islands of Trinidad and Tobago and neighboring regions in South America. Male guppies exhibit highly polymorphic and genetically variable secondary sexual coloration.

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tion consisting of carotenoid, drosoperin, and melanin pigments and structural colors (Houde 1997; Grether et al. 2001; Hughes et al. 2005). The saturation (chroma) and area of male orange spots, in addition to overall color contrast, tail area, and a host of other traits, are correlated with male attractiveness and mating success (Kodric-Brown 1989; Endler and Houde 1995; Grether 2000; Brooks and Endler 2001). Furthermore, additive genetic variation exists in most of these traits, and thus, they are responsive to natural and sexual selection (Brooks and Endler 2001). Females prefer the most conspicuous males, yet these males are also the most apparent to predators, so that an equilibrium between sexual selection and predation is achieved in each population (reviewed by Endler 1995; Houde 1997). Female preferences vary among populations, specifically in the subset of traits females use to assess males and in the magnitude and sign of their preference for a particular trait (Endler and Houde 1995). However, orange coloration is preferred in most populations (Endler and Houde 1995). While females receive no direct benefits from mate choice, they do incur a predation cost for this choice (Godin and Briggs 1996), suggesting that preferences are sustained through the indirect benefits of good genes (Pomiankowski 1987).

In support of the Hamilton–Zuk hypothesis, parasitic infection induced in laboratory guppies increased the lightness and decreased the saturation of a male's orange spots for at least 1 day after the parasites were successfully removed (Houde and Torio 1992). In addition, female guppies preferred uninfected males over the previously infected males with less saturated, paler orange spots (Houde and Torio 1992). Moreover, males with lower parasite loads had higher courtship display rates, and females preferred these males (Kennedy et al. 1987; McMinn 1990), as well as males that acquired parasite resistance after infection (López 1998). In the field, variation in parasite resistance between populations has been demonstrated (Oosterhout et al. 2003).

Gyrodactylus parasites, which are ectoparasitic monogenean flatworms, occur at high frequency in wild guppy populations (Harris and Lyles 1992; Oosterhout et al. 2003). Two species, *Gyrodactylus turnbulli* and *Gyrodactylus bullatarudis*, are highly pathogenic and infect approximately 50% of wild guppies in some populations (Harris and Lyles 1992; Oosterhout et al. 2003). Scott and Anderson (1984) measured a daily mortality rate of 7% in guppies infected with *G. turnbulli* relative to 0.4% in uninfected fish. Thus, this parasite sufficiently reduces fitness to drive female choice as Hamilton and Zuk (1982) stipulated. Moreover, it is not so harmful that it automatically prevents infected males from reproducing. Thus, females are still faced with a choice between infected and uninfected males (McLennan and Brooks 1991).

The saturation and lightness of orange spots are largely condition-dependent in guppies, rather than genetic (Houde 1997), and thus have the potential to signal male quality, such as parasite resistance (Kodric-Brown and Brown 1984). Animals are unable to synthesize carotenoid pigments (Goodwin 1984), a major component of orange spots in guppies, and thus, male guppies must be efficient foragers to attain good coloration and attract females (Endler 1980; Endler 1983). Males provided a carotenoid-rich diet showed increased brightness, saturation, and development of their orange spots (Kodric-Brown 1989; Grether 2000; Karino and Haijima 2004). Secondly, carotenoids enhance the guppy immune system (Grether et al. 2003). Males on a carotenoid-rich diet showed an increased acquired immune response suggesting that males may be faced with a tradeoff between allocating carotenoids to sexual coloration or to immune function (Folstad and Karter 1992; Grether et al. 2003).

Most studies find the total area of a male's orange spots, however, to be a genetic trait with no environmental component (Kodric-Brown 1989; Houde 1997; but see Karino and Shinjo 2004). Although orange area was found to correlate with swimming performance (Nicoletto 1993), this could indicate heritable genetic constitution or condition dependence. Thus, orange area (and its subdivision into number of orange spots) should only be reflective of evolutionary trends in a population or a male's genetic quality. On the other hand, condition-dependent traits, such as saturation and lightness of orange spots, can reflect either environmental components of an individual's current fitness or genetic differences in acquiring or retaining carotenoid pigments.

This study tests two key predictions of the Hamilton–Zuk hypothesis: (1) that within a population, males with the most prominent (most intense, saturated, numerous, or largest) orange spots have the fewest parasites and (2) that in populations with the greatest number of parasites, males have the most prominent (as above) orange spots on average. To sample a wide environmental gradient, male guppies were collected from both high-predation and low-predation environments, inspected for parasites, and photographed. The color, number, and size of orange regions was then measured and analyzed relative to the Hamilton–Zuk predictions.

Materials and methods

Sampling

A total of 568 male guppies from 19 populations were collected by net in the Northern Range of Trinidad in August 2004 (see Appendix for population details). Populations

were separated by at least 1 km, a conservative upper bound of gene flow distance (Reznick and Endler 1982). In high-elevation, low-predation sites, guppies were collected from one to three adjacent pools per population, while low-elevation streams were not subdivided into pools, and guppies were collected along 10–100 m stretches. The total number of each sex was recorded, and all females were released. Males were anesthetized in a 0.2% solution of tricaine methanesulfonate (MS-222; Argent Laboratories, Redmond, WA, USA) and immediately inspected for parasites under a dissection scope. *G. turnbulli* could not be differentiated from *G. bullatarudis*, and so, the total number of *Gyrodactylus* spp. parasites on each fish was recorded. Other taxa of external parasites were observed rarely ($N=5$) and so were not included in the parasite load. Each male was then photographed against a black background along with a standard orange color patch (x, y, Y coordinates [0.41, 0.38, 53] under standard illuminant D65). All males were allowed to recover from anesthesia and then released at their capture site. Nets and holding containers were sterilized before use at another population to prevent pathogen transfer. Limited parasite transfer might have been possible among males within a population during the brief period spent in the same container before parasite inspection. However, this would not increase the total parasite count in a population because males were moved to a separate recovery container after inspection. Secondly, most parasite transfer probably occurs during the period of male–female copulation (López 1998) and no females were added to the male holding container.

Predation intensity was characterized as low or high for each site based on location and previous literature (Appendix). High-predation sites contained the voracious guppy predator, *Crenicichla alta* (pike cichlid), in addition to other predators such as *Aequidens pulcher* (blue acara) and several characin species (Endler 1978). High-predation status was determined by location within the altitude range of this predator fauna as described by Endler (1978) and by published descriptions of some sites in this study (Reznick and Endler 1982; Endler and Houde 1995). The Hollis Reservoir (*Hollis*) was considered a high-predation site due to introductions of piscivorous fish. Low-predation sites contained only the killifish *Rivulus hartii*, a predator of immature guppies, and, in one case (*Paria 4*), the prawn *Macrobrachium crenulatum*, which has been observed to prey on adult guppies (Endler 1978). Previous studies (Reznick and Endler 1982; Endler and Houde 1995; Rodd et al. 2002) assigned low-predation status to the sites in this study.

Image analysis—hue, saturation, lightness of orange spots

All orange spots on one lateral surface of each fish, including tail and dorsal fins, were measured. The average

hue (red-orange-yellow; ranging from 305 through 0 to 55° on a 360° scale), saturation (0–100% range; i.e., color purity), and lightness (0–100% range; brightness, reflectance, or value; hue, saturation, and lightness referred to hereafter as HSL) of three points on each spot were measured using image analysis software (Photoshop 7.0, Adobe Systems, San Jose, CA, USA), similar to the methods of Karino and Haijima (2001, 2004). The saturation and lightness values obtained for each guppy were divided by the values of the orange reference patch in the same image, resulting in normalized values that accounted for variation in illumination and focus among images. Hue was first converted to a continuous variable by adding 360° to values between 0 and 55° and then normalized by subtracting the hue of the orange reference patch in each image. We chose image analysis over spectrometry for its suitability to the field. Neither method is superior based on current knowledge of guppy perception (Grether et al. 2005).

Image analysis—area and number of orange spots

The combined area of all orange spots and the lateral surface area of each fish were measured in the following manner. The scale of each image was determined using a length standard in the background. The boundaries of each orange spot on one lateral surface were first traced by eye. The enclosed area was then measured using Scion Image 4.0.3.2 (Scion, 2001; based on NIH Image for Macintosh). Finally, all spots outside the red-orange hue range (305 through 0 to 55°) were excluded. The sum area of all the spots was divided by the area of the same lateral surface of the fish (measured in the same way, including tail) to determine the relative orange area. The number of orange spots (defined as above) on this surface was also recorded.

Data analysis

Eight measures of a male's orange ornamentation were used to test both the within-population and among-population predictions of the Hamilton–Zuk hypothesis. Relative orange area, number of orange spots, and average HSL values of all orange spots on one lateral surface of each fish were measured as described above. The HSL values and area of each spot were also used to calculate area-weighted HSL measures. These area-weighted measures estimate the total carotenoid investment of each male in his orange spots. Average HSL values of each spot were first multiplied by the relative area of that spot. This procedure accounts for different contributions of different spot sizes to the total HSL values of each fish. These area-weighted HSL values for each spot were then averaged for each fish.

The within-population prediction was tested by searching for a correlation between parasite load and the eight measures of male ornamentation. This correlation occurs on the individual level. The correlation was tested in two ways. First, a hierarchical approach was used to detect the correlation within all populations. Three-way analyses of variance (ANOVAs) with the effects of drainage (Appendix), population nested within drainage, and parasite load nested within population and drainage were performed for all eight measures of male ornamentation (Fig. 1, Table 1). This analysis was performed separately for low-predation and high-predation environments. Parasite load could not be transformed to meet parametric assumptions and so was modeled as an ordinal variable. Parasite load was also included as a binary variable (0 parasites or >0 parasites) in a separate analysis to increase statistical power. The effect of drainage was included to control for any possible nonindependence of sample sites. The effect of population nested within drainage was included to account for additional ecological variation beyond predation intensity. Population nested within drainage was included as a fixed effect. This procedure allowed us to estimate and test differences in parasite load among populations. Treating population as a random effect would reduce the degrees of freedom for significance tests of drainage effects and, thereby, produce a more conservative test (KA Hughes, personal communication). However, drainage effects were not of direct interest in this study.

For a second test of the within-population prediction, the *Mausica A* population was analyzed separately due to its extremely high prevalence of parasites (in addition to

inclusion in high-predation populations for the above analysis). All eight measures of *Mausica A* male ornamentation were compared with a male's parasite load (Fig. 1, Table 2). Parasite load was tested both as a continuous and a binary variable.

The among-population prediction was tested by searching for a correlation between parasite abundance and a male's ornamentation on the population level. Parasite abundance in each population was estimated from the *Gyrodactylus* parasite load on male fish at each site (Fig. 2). Three-way analyses of covariance (ANCOVAs) with the effects of drainage (Appendix), population nested within drainage, and parasite load were performed for all eight measures of male ornamentation (Fig. 1, Table 3). This analysis was performed separately for low-predation and high-predation environments.

In addition to testing the two central questions of the study, the relationships of two ecological correlates with parasite load, predation intensity and surface area were tested. Non-normal data were analyzed using the Wilcoxon nonparametric test (Fig. 1, Table 2) or modeled as ordinal variables (Fig. 1, Tables 1 and 3). Only three response variables could be transformed to meet normality assumptions (relative area, hue, and saturation; $\log [C \pm x]$ where C denotes an appropriate constant to balance the distribution; two outliers were excluded from saturation, 441.8 and 36.3%). However, the results were qualitatively the same using these transformed variables or untransformed data in parametric analyses.

Retrospective power analyses were performed in two ways. First, observed power was computed for all response

Fig. 1 Graphical depiction of the statistics used to test both the within-population and among-population predictions of the Hamilton–Zuk hypothesis. **a** Three-way ANOVAs with the effects of drainage (Appendix), population nested within drainage, and parasite load nested within population and drainage were performed for all eight measures of male ornamentation (Table 1). **b** Within the *Mausica A* population, the eight measures of male ornamentation were each compared with a male's parasite load (Table 2). **c** Three-way ANCOVAs with the fixed effects of drainage (Appendix), population nested within drainage, and parasite load were performed for all eight measures (Table 3). Analyses of **a** and **c** performed separately for low-predation and high-predation environments

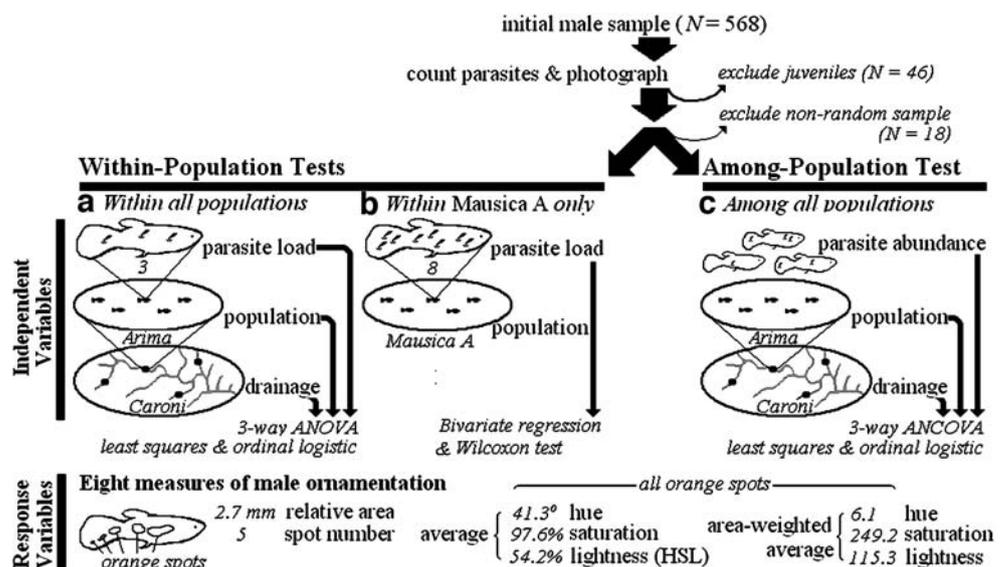


Table 1 Statistics for tests of the within-population predictions of the Hamilton–Zuk hypothesis conducted for all populations in this study

Test	Parasite variable ^a	Response variable	High predation					Low predation				
			N ^e	F/ χ^2 ^d	df	Obs. power ^e	P	N ^f	F/ χ^2	df	Obs. power	P
Within-population	Parasite load (ordinal)	Hue	357	0.606 _F	41	0.68	0.973 _{D,P}	123	0.993 _F	6	0.38	0.434 _{D,P}
		Saturation	356	1.098 _F	41	0.96	0.323 _{D,P}	122	0.533 _F	6	0.21	0.782 _D
		Lightness	357	0.995 _F	41	0.93	0.484 _{D,P}	123	0.815 _F	6	0.31	0.560
		Rel. area	357	0.832 _F	41	0.86	0.759 _D	124	1.075 _F	6	0.41	0.382 _D
		Spot num.	357	42.81 χ^2	41	Power ^g	0.393 _{D,P}	124	2.310 χ^2	6	Power	0.889
		Wtd. H ^b	357	45.30 χ^2	41	$f_{0.15}^2$ 0.20	0.297 _{D,P}	123	3.402 χ^2	6	$f_{0.15}^2$ 0.18	0.757 _{D,P}
	Infection status (binary)	Wtd. S ^b	357	49.79 χ^2	41	$f_{0.35}^2$ 0.95	0.163 _D	123	6.821 χ^2	6	$f_{0.35}^2$ 0.82	0.338 _{D,P}
		Wtd. L ^b	357	41.10 χ^2	41		0.466	123	6.090 χ^2	6		0.413 _{D,P}
		Hue	357	0.682 _F	12	0.40	0.769 _P	123	0.428 _F	4	0.15	0.788 _D
		Saturation	356	1.113 _F	12	0.64	0.348 _{D,P}	122	0.436 _F	4	0.15	0.783 _D
		Lightness	357	1.294 _F	12	0.73	0.220 _{D,P}	123	1.213 _F	4	0.37	0.309
		Rel. area	357	0.728 _F	12	0.43	0.724 _D	124	0.798 _F	4	0.25	0.529
		Spot num.	357	12.77 χ^2	12	Power	0.386 _{D,P}	124	1.997 χ^2	4	Power	0.736
		Wtd. H ^b	357	5.884 χ^2	12	$f_{0.15}^2$ 0.39	0.922 _{D,P}	123	0.947 χ^2	4	$f_{0.15}^2$ 0.22	0.918 _{D,P}
		Wtd. S ^b	357	10.74 χ^2	12	$f_{0.35}^2$ 0.99	0.551	123	3.245 χ^2	4	$f_{0.35}^2$ 0.87	0.517 _{D,P}
		Wtd. L ^b	357	11.12 χ^2	12		0.518	123	5.505 χ^2	4		0.239 _{D,P}

Subscripts of D and P indicate a significant effect of drainage and/or population in the model.

Rel. area Relative orange area: summed area of all orange spots on one lateral surface of the male divided by the area of one lateral surface, including tail. *Wtd. HSL* area-weighted hue, saturation, and lightness

^a Infection status (binary): (0 parasites or >0 parasites)

^b Wtd. HSL measures account for the relative contributions of different spot sizes to the total HSL values of each fish. First, the normalized HSL values of each spot were multiplied by the respective area of each spot relative to one lateral surface of the male. The area-weighted HSL values of each spot were then averaged to obtain the area-weighted HSL values for each male. For example: $A_w = \frac{1}{N} \cdot \frac{1}{A_{\text{fish}}} \sum_{n=1}^N \text{hue}(S_n) \cdot \text{area}(S_n)$, Where N is the number of spots, S_n is one spot, and A_{fish} is the area of one lateral surface of the fish.

^c We searched for additional males with a high parasite count in some populations ($N=18$). These males were only included in within-population analyses. Parasite abundances (Fig. 2) and among-population analyses (Fig. 1, Table 3) used a random sample of males.

^d Rel. area, hue, saturation, and lightness were modeled as continuous variables in a least squares regression, and the F statistic is reported. Relative area, hue, and saturation were transformed to meet normality assumptions ($\log [C \pm x]$ where C denotes an appropriate constant to balance the distribution; two outliers were excluded from saturation, 441.8 and 36.3%), and lightness was distributed normally (Shapiro–Wilk test; $P>0.05$). Spot number and area-weighted HSL could not be transformed to meet normality assumptions and were modeled as ordinal variables in a logistic regression. The nonparametric Wald χ^2 test statistic is reported.

^e An observed power analysis was performed for all continuous variables (excluding spot number and area-weighted HSL modeled as ordinal variables in this table). Power ($1-\beta$ error) represents the probability of rejecting the null hypothesis, given that the alternative hypothesis is true. We computed observed power using the root mean square error of the model for the variance and the observed effect size: $\sqrt{\frac{SS_h}{N}}$, Where SS_h is the sum of squares for the hypothesis, and N is the sample size.

^f One low-predation male without orange spots was excluded from all HSL analyses.

^g We also provide a power analysis based on standardized medium (Cohen's $f^2=0.15$) and large ($f^2=0.35$) effect sizes (Cohen 1988). These power estimates are independent of the sampling variance and observed effect size and, thus, are provided for each statistical model, rather than each response variable.

variables in Tables 1, 2, and 3 using the observed variance and effect size. However, this method has been criticized, as it provides no additional information beyond the P value, and observed effect sizes may be a poor estimate of population parameters (Thomas 1997). Thomas (1997) recommends using a standardized effect size, independent of sample variance, for complex analyses. Therefore, we also used the standardized medium ($f^2=0.15$) and large ($f^2=0.35$) effect sizes of Cohen (1988) to compute the power of the model in Tables 1, 2, and 3. Although these measures are not biologically meaningful, they allow comparison of the different statistical models used in this study.

All tests were performed using JMP-IN 5 (SAS Institute, Cary, NC, USA) and G*Power (Faul et al. 2007). Males

without fully developed sexual organs ($N=46$) were excluded from all analyses.

Results

Parasite load

The average number of *Gyrodactylus* parasites per male guppy was 0.68 ± 0.08 (mean \pm SE) and population averages ranged from 0 to 2.8 parasites per male (Fig. 2; Appendix). The number of parasites on each fish ranged from 0 to 10, with one outlier having 28 parasites. Males with more than

five parasites were only found in the Mausica A and Valencia populations, with Mausica A males having an unusually high prevalence of parasites overall.

Tests of within-population and among-population predictions of the Hamilton–Zuk hypothesis

The orange spots of males infected by at least one *Gyrodactylus* were not significantly different in HSL, area-weighted HSL, area, or number of orange spots from uninfected males within high- or low-predation environments (Table 1; Fig. 3). There was also no difference in these eight measures of male ornamentation when parasite load was included as a continuous variable in the models (Table 1). Within the most heavily parasitized Mausica A population, there was no difference between infected and uninfected males in the eight measures of male ornamentation (Wilcoxon tests; Table 2). Nor was there a difference

when parasite load was included as a continuous variable (Table 2). Among populations, parasite load had no significant effect on all eight measures of male orange ornamentation (Fig. 4; Table 3).

Differences in male ornamentation between low-predation and high-predation environments

Fish in high-predation environments possessed a lower number of orange spots ($Z=3.970$, $df=1$, $P<0.0001$), and these spots were less saturated ($Z=4.871$, $df=1$, $P<0.0001$) and lighter ($Z=-6.746$, $df=1$, $P<0.0001$) than fish in low-predation environments (Wilcoxon tests; Fig. 3). Area-weighted orange spots were less saturated in high-predation environments (Wilcoxon test; $Z=3.482$, $df=1$, $P=0.0005$) with no significant difference in lightness. Hue, area-weighted hue, and relative orange areas were not significantly different between high- and low-predation environments.

Table 2 Statistics for tests of the within-population predictions of the Hamilton–Zuk hypothesis conducted within only the Mausica A population

Test	Parasite variable ^a	Response variable	High predation					
			<i>N</i>	<i>t/Z</i> ^c	<i>df</i>	<i>r</i> ² /Obs. power ^d	<i>P</i>	Power ^e
Within Mausica A population	Parasite load (continuous)	Hue	65	0.80	1	0.010	0.427	
		Saturation	65	0.91	1	0.013	0.365	
		Lightness	65	0.79	1	0.010	0.435	
		Rel. area	65	0.69	1	0.008	0.492	
		Spot num.	65	-1.34	1	0.028	0.185	
		Wtd. H ^b	65	-0.39	1	0.002	0.696	
		Wtd. S ^b	65	1.91	1	0.055	0.061	
	Wtd. L ^b	65	1.95	1	0.057	0.056		
	Infection status (binary)	Hue	65	-1.548 _t	1	0.27	0.153	
		Saturation	65	-0.693 _t	1	0.12	0.508	$f_{0.15}^2 0.23^f$
		Lightness	65	0.977 _t	1	0.11	0.347	$f_{0.35}^2 0.84^f$
		Rel. area	65	-1.429 _t	1	0.26	0.185	
		Spot num.	65	-0.804 _Z	1	0.13	0.421	
		Wtd. H ^b	65	-1.847 _Z	1	0.55	0.065	$f_{0.15}^2 0.06^g$
Wtd. S ^b		65	0.849 _Z	1	0.11	0.396	$f_{0.35}^2 0.12^g$	
Wtd. L ^b	65	0.669 _Z	1	0.09	0.504			

Rel. area Relative orange area: summed area of all orange spots on one lateral surface of the male divided by the area of one lateral surface, including tail. *Wtd. HSL* area-weighted hue, saturation, and lightness

^a Infection status (binary): (0 parasites or >0 parasites)

^b *Wtd. HSL* measures account for the relative contributions of different spot sizes to the total HSL values of each fish. First, the normalized HSL values of each spot were multiplied by the respective area of each spot relative to one lateral surface of the male. The area-weighted HSL values of each spot were then averaged to obtain the area-weighted HSL values for each male. For example: $A_w = \frac{1}{N} \cdot \frac{1}{A_{fish}} \sum_{n=1}^N \text{hue}(S_n) \cdot \text{area}(S_n)$, Where N is the number of spots, S_n is one spot, and A_{fish} is the area of one lateral surface of the fish.

^c The t test was performed for normally-distributed variables and the Wilcoxon test (Z statistic) was performed for non-normal variables.

^d An observed power analysis was performed for all continuous variables in this table. Power ($1-\beta$ error) represents the probability of rejecting the null hypothesis, given that the alternative hypothesis is true. We computed observed power using the root mean square error of the model for the variance and the observed effect size: $\sqrt{\frac{SS_h}{N}}$,

Where SS_h is the sum of squares for the hypothesis, and N is the sample size.

^e We also provide a power analysis based on standardized medium (Cohen's $f^2=0.15$) and large ($f^2=0.35$) effect sizes (Cohen 1988). These power estimates are independent of the sampling variance and observed effect size and, thus, are provided for each statistical model, rather than each response variable.

^f Standardized power for t test

^g Standardized power for Wilcoxon test

Table 3 Statistics for all tests of the among-population predictions of the Hamilton–Zuk hypothesis conducted in this study

Test	Parasite variable ^a	Response variable	High predation					Low predation				
			<i>N</i>	F/χ^2 ^c	<i>df</i>	Obs. power ^e	<i>P</i>	<i>N</i> ^e	F/χ^2	<i>df</i>	Obs. power ^d	<i>P</i>
Among-populations	Parasite load (ordinal)	Hue	339	0.591 _F	10	0.31	0.821 _{D,P}	123	2.316 _F	2	0.46	0.103 _{D,P}
		Saturation	338	1.485 _F	10	0.74	0.144 _{D,P}	122	0.219 _F	2	0.08	0.804 _D
		Lightness	339	1.438 _F	10	0.72	0.162 _{D,P}	123	0.681 _F	2	0.16	0.508
		Rel. area	339	1.086 _F	10	0.57	0.372 _D	124	1.146 _F	2	0.25	0.321 _{D,P}
		Spot num.	339	7.673 χ^2	10	Power ^g	0.661 _{D,P}	124	1.013 χ^2	2	Power	0.603 _P
		Wtd. H ^b	339	9.342 χ^2	10	$f^2_{0.15}$ 0.40	0.500 _{D,P}	123	2.293 χ^2	2	$f^2_{0.15}$ 0.29	0.318 _{D,P}
		Wtd. S ^b	339	10.01 χ^2	10	$f^2_{0.35}$ 0.99	0.440 _{D,P}	123	2.435 χ^2	2	$f^2_{0.35}$ 0.94	0.296 _{D,P}
		Wtd. L ^b	339	7.078 χ^2	10		0.718 _P	123	5.119 χ^2	2		0.077 _{D,P}

Subscripts of D and P indicate a significant effect of drainage and/or population in the model.

Rel. area Relative orange area: summed area of all orange spots on one lateral surface of the male divided by the area of one lateral surface, including tail. *Wtd. HSL* area-weighted hue, saturation, and lightness

^a Infection status (binary): (0 parasites or >0 parasites)

^b Wtd. HSL measures account for the relative contributions of different spot sizes to the total HSL values of each fish. First, the normalized HSL values of each spot were multiplied by the respective area of each spot relative to one lateral surface of the male. The area-weighted HSL values of each spot were then averaged to obtain the area-weighted HSL values for each male. For example: $A_w = \frac{1}{N} \cdot \frac{1}{A_{\text{fish}}} \sum_{n=1}^N \text{hue}(S_n) \cdot \text{area}(S_n)$, Where *N* is the number of spots, *S_n* is one spot, and *A_{fish}* is the area of one lateral surface of the fish.

^c Rel. area, hue, saturation, and lightness were modeled as continuous variables in a least squares regression, and the *F* statistic is reported.

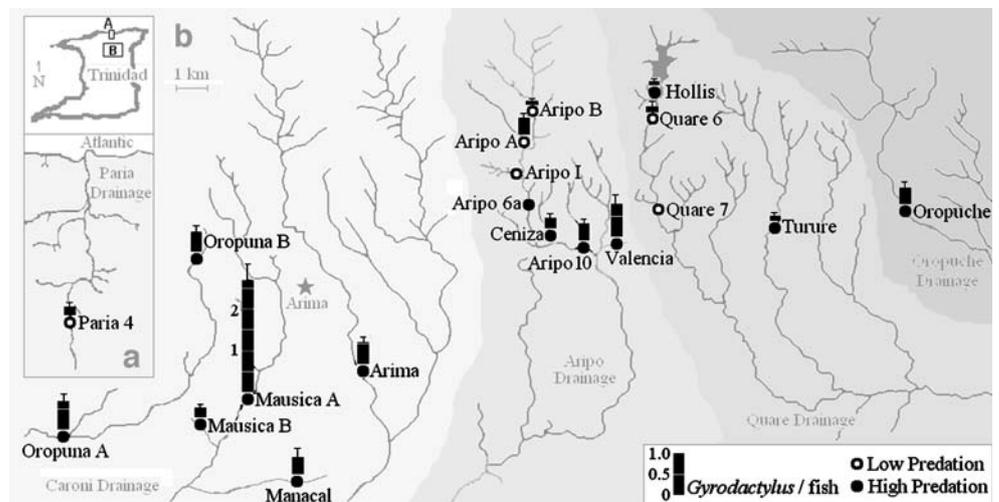
Relative area, hue, and saturation were transformed to meet normality assumptions [$\log(C \pm x)$ where *C* denotes an appropriate constant to balance the distribution; two outliers were excluded from saturation 441.8% and 36.3%) and lightness was distributed normally (Shapiro-Wilk test; $P > 0.05$). Spot number and area-weighted HSL could not be transformed to meet normality assumptions and were modeled as ordinal variables in a logistic regression. The nonparametric Wald χ^2 test statistic is reported.

^d An observed power analysis was performed for all continuous variables (excluding spot number and area-weighted HSL modeled as ordinal variables in this table). Power ($1 - \beta$ error) represents the probability of rejecting the null hypothesis, given that the alternative hypothesis is true. We computed observed power using the root mean square error of the model for the variance and the observed effect size: $\sqrt{\frac{SS_h}{N}}$, Where *SS_h* is the sum of squares for the hypothesis, and *N* is the sample size.

^e One low-predation male without orange spots was excluded from all HSL analyses.

^f We also provide a power analysis based on standardized medium (Cohen's $f^2 = 0.15$) and large ($f^2 = 0.35$) effect sizes (Cohen 1988). These power estimates are independent of the sampling variance and observed effect size and, thus, are provided for each statistical model in this table, rather than each response variable.

Fig. 2 *Gyrodactylus* parasites per male guppy (mean+SE) in all populations and their geographic location



Ecological correlates of parasite load: predation intensity and lateral surface area

High-predation environments contained significantly more parasites than low-predation environments ($N_{\text{high}}=393$, $N_{\text{low}}=153$, $Z=-6.300$, $df=1$, $P<0.0001$; Wilcoxon test) with means of 0.85 ± 0.10 and 0.16 ± 0.05 *Gyrodactylus* per male, respectively. To control for greater sampling effort in high-predation environments, a random sample from high-predation environments was selected equal to the sample size of low-predation environments and reanalyzed. Parasite load remained significantly higher in high-predation environments ($N=304$, $Z=-6.262$, $df=1$, $P<0.0001$; Wilcoxon test). There was no difference in lateral surface area between infected and uninfected fish in high-predation (infected 60 ± 1.2 , uninfected 60 ± 0.8 mm²) or low-predation environments (infected 82 ± 5.1 , uninfected 76 ± 1.3 mm²). There was a significant difference ($Z=10.483$, $df=1$, $P<0.0001$; Wilcoxon test) in lateral surface area between high-predation (59.96 ± 0.70) and low-predation (76.75 ± 1.30) environments.

Discussion

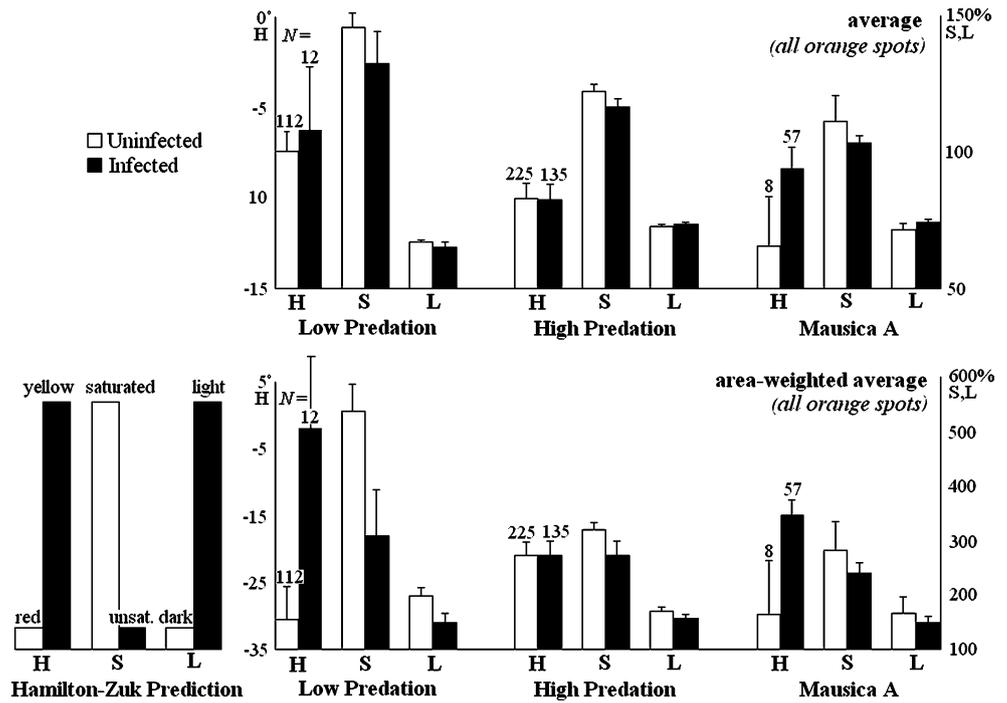
This study found no support for the within-population and among-population predictions of the Hamilton–Zuk hypothesis. The greatest selective pressure for females to choose resistant males would presumably be within the most heavily parasitized population (Mausica A) in this study. Nonetheless, there was no relationship between male ornamentation and parasite load even exclusively within this population. In contrast to this field study, the laboratory study of orange coloration from infected guppies by Houde and Torio (1992) was consistent with the within-population prediction of the Hamilton–Zuk hypothesis. One day after all parasites were successfully removed, the orange coloration of male guppies previously infected with *G. turnbulli* was lighter and less saturated than that of uninfected individuals (Houde and Torio 1992). Only 24 matched pairs were measured for Houde and Torio’s laboratory study. Thus, the power of this field study sampling more than 500 fish was likely sufficient to detect a similar effect. However, consistent with the present study, López (1998) found no effect of *G. turnbulli* parasites on the saturation of male orange spots 10 days after recovery from infection. These two experimental studies suggest male guppies require between 1 and 10 days to recover their original orange saturation level after an initial parasite infection. If male colors remain affected after recovery from a parasite infection, observational field studies cannot distinguish resistant males that have never been infected from nonresistant males that do not currently harbor any para-

sites but still exhibit reduced coloration from a previous infection. As a result, the overall coloration of uninfected males measured in any observational field study will be lower than the coloration of resistant males. Nonetheless, they will be classed in the same group of “uninfected males.” Thus, it is more difficult for observational field studies to detect differences in coloration between resistant and nonresistant males. However, this confounding factor is only significant if a large number of recovering males (most likely males recovering from an infection within 10 days previous) are present in the population.

Additional traits may signal male parasite resistance to females. Male guppies infected with *Gyrodactylus* displayed to females at a lower rate than uninfected males (Kennedy et al. 1987). Secondly, resistant males displayed at a higher rate than nonresistant males after recovering from an initial *Gyrodactylus* infection but showed no differences in orange saturation levels (López 1998). Dynamic traits, such as display rate, are capable of changing rapidly to express a male’s current condition, while static traits, such as coloration, require hours or days (Kodric-Brown and Nicoletto 2001). Guppy display rates might convey more information to females than orange coloration, for example, the information that a male has acquired resistance in López’s study. However, dynamic traits most likely require more time than static traits to sufficiently assess and thus incur a higher predation cost to females. Females could be faced with a tradeoff between informational content and assessment time in allocating their attention to the available male indicator traits. Nonetheless, females were evenly divided in their preference for a male with an attractive display (and no color) or a colorful male (with low display rate) in controlled binary choice trials (Kodric-Brown and Nicoletto 2001). More work is needed on the relative informational content among multiple traits and the costs to females for choosing among these traits.

Finally, Getty (2002) recently pointed out that all tests of the within-population prediction of the Hamilton–Zuk hypothesis are making two unfounded assumptions. First, parasite load may not always indicate poor male condition; high quality males may have a higher tolerance and could harbor more parasites than low quality males. Nevertheless, we also found no support in this study for more highly ornamented males harboring more parasites than less ornamented males. Secondly, it has traditionally been assumed that high quality, resistant males pay a lower cost for a colorful display to females than low quality, nonresistant males. However, high quality males might pay an equal or higher cost in the reduction in their immunocompetence for a colorful display relative to low quality males. High quality males might then carry an equal or greater number of parasites (Getty 2002). Thus, it may

Fig. 3 Normalized hue (*H*), saturation (*S*), and lightness (*L*) values (mean±SE) of orange coloration in infected and uninfected males in low-predation and high-predation (including Mausica A) environments and within the Mausica A (high-predation) population. Test of the within-population prediction of the Hamilton–Zuk hypothesis. Infected represents males carrying at least one *Gyrodactylus*. Sample sizes (*N*) for uninfected and infected fish are depicted above the respective bars for each group

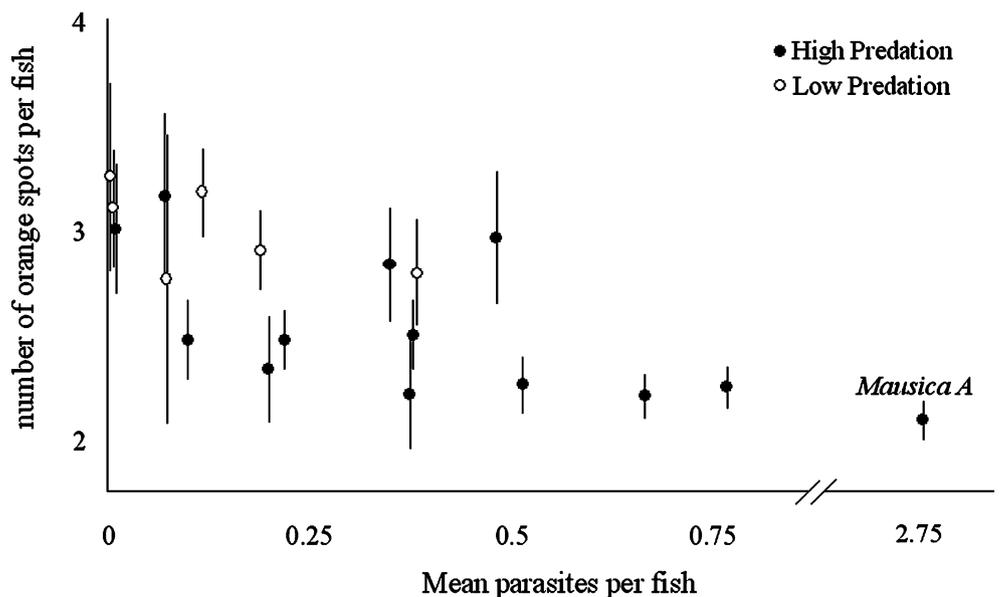


be futile for observational studies, such as this one, to test the within-population prediction of the Hamilton–Zuk hypothesis by measuring parasite loads and only one measure of male quality.

This study also addressed the second, among-population prediction of the Hamilton–Zuk hypothesis: that populations subjected to the greatest fitness loss due to parasites during their history should evolve the most extravagant sexual traits. Traditionally, this prediction has been tested

by surveying the showiness of congeneric species and the species richness of parasite taxa in their respective habitats. In this study, we surveyed populations of a single species and measured the abundance of a single parasite genus (by far the most common) on males at each site as a proxy for parasite exposure. The amount of male ornamentation in a population reflects the strength of sexual selection in the environment. Thus, male ornamentation should increase in environments with more abundant or more harmful para-

Fig. 4 Number of orange spots (mean±SE) vs average *Gyrodactylus* parasites per male guppy (SE bars not shown to maintain clarity) across all populations. Test of the among-population prediction of the Hamilton–Zuk hypothesis



sites where females have increased selection pressure to choose resistant males. Populations were separated by at least 1 km, a conservative estimate of the distance between genetically distinct populations (Reznick and Endler 1982). Marked life-history evolution has been demonstrated in this species over short timescales in populations separated by a small distance (Reznick et al. 1997), thus populations are likely to exhibit different evolutionary trajectories. Moreover, drainage effects were controlled for in the statistical model. Finally, host resistance and parasite fitness may vary at each site, so measuring the number of parasites on each host is not ideal. However, this method is simpler than attempting to measure the abundance or diversity of parasites in the environment or quantifying host resistance and parasite virulence at each site.

We found no evidence for the among-population Hamilton–Zuk prediction that selection from parasites increases male sexual ornamentation. Numerous caveats have been proposed for testing this prediction (Endler and Lyles 1989; Schall and Staats 1997; Møller et al. 1999), and this study addressed these warnings by controlling for the predation level and potential nonindependence of sample populations among drainages, censusing a stable parasite population that significantly affects host mortality (Scott and Anderson 1984; Harris and Lyles 1992), using only objective measures of coloration, and sampling from widespread populations.

Two confounding factors of the among-population prediction unmeasured in this study were the carotenoid availability and turbidity of each stream. Guppies in streams with greater carotenoid availability can more easily find sufficient food to maintain the coloration of their orange spots, thus reducing the value of this trait as an indicator of male foraging ability to females (Grether 2000). The two populations with the highest parasite abundance in this survey by far, Mausica A and Valencia, were highly disturbed. Both streams drained urban areas and algae covered virtually all surfaces, indicative of human wastewater. High carotenoid availability, rather than high parasite abundance, could increase the saturation and brightness of male orange coloration in these populations and confound the among-population test. Similarly, turbidity also increases in downstream, high-predation environments. Male guppies may have to increase the brightness of their coloration to be seen by females in turbid environments. Nevertheless, as this study found no such increase in high-predation populations, differing carotenoid availability among streams is a moot point.

One additional finding in this study is that high-predation populations have a far higher abundance of

Gyrodactylus infections. This has been reported previously (Lyles 1990), and Endler (1995) suggested that tighter schooling in high-predation environments might allow easier transmission of parasites, despite the lower densities of guppies in high-predation environments (Endler 1978; Endler 1995). Higher infection rate is confounded by lower elevation, higher temperature, greater human disturbance, and a plethora of life history changes (Endler 1995) in high-predation environments. Fortunately, high-predation sites were sampled from both the Caroni (west-draining) and the Oropuche (east-draining) river systems in which guppies differ in their schooling tendencies (Magurran 1999). Guppies from high-predation sites in the Oropuche system school less often than their Caroni counterparts, suggesting that they should have a lower infection rate. In this study, Oropuche males were not significantly more infected in a two-way ANOVA with the effect of river system (Caroni or Oropuche) and population nested within river system (results not presented). Although not significant, the difference was in the expected direction in support of the schooling hypothesis: 0.96 ± 0.11 parasites per fish in Caroni sites (tighter schooling) compared to only 0.23 ± 0.10 parasites in Oropuche sites.

Finally, higher parasite infection in high-predation populations may be consistent with the Hamilton–Zuk hypothesis. In high-predation environments, female choice is counteracted by the need for increased predator vigilance (Godin and Briggs 1996), more sneaky copulations by males (Luyten and Liley 1985), and reduced visibility (Luyten and Liley 1991), thereby weakening the strength of sexual selection for parasite-resistant males if the hypothesis holds. One prediction of this explanation is that guppies from low-predation populations should be more resistant to parasites than guppies from high-predation populations. However, Oosterhout et al. (2003) found the opposite result: A single low-predation population recovered from infection with a common *Gyrodactylus* strain more slowly than a high-predation population from the same river (Oosterhout et al. 2003). Increased gene flow and exposure to more diverse parasite populations in downstream high-predation populations may explain this outcome (Oosterhout et al. 2003). Nonetheless, low-predation fish, although more vulnerable, should evolve resistance more quickly to novel parasites through increased selection for resistant males by females if the Hamilton–Zuk hypothesis holds.

In conclusion, this field test of guppies found no evidence for the within- and among-population predictions of the Hamilton–Zuk hypothesis, despite previous laboratory support for this idea. We tested eight measures of a

male's orange ornamentation: hue, saturation, lightness, area, number of spots, and area-weighted hue, saturation, and lightness. We found no relationship between these measures and a male's parasite load within populations or the overall parasite abundances among populations. Nonetheless, Hamilton–Zuk predictions for the within-population test remain unclear without further understanding of the costs and benefits of male-signaling, and the among-population test may have been confounded by additional ecological variables, such as carotenoid availability. Therefore, further work testing the Hamilton–Zuk hypothesis in this system would be worthwhile. Finally, guppies in high-predation environments carried significantly greater numbers of parasites, possibly due to tighter schooling density or relaxed female mate choice.

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Appendix

Table 4 Data for populations sampled

Site ^a	Trinidad National Grid Coordinates ^b	Predation intensity ^c	Predator fauna ^d	Drainage ^e	Total sample ^f	Females/males ^g	Gyrodactylus abundance ^h
Aripo A	1,181 N, 694 E	Low ¹	R	Aripo ^C	26	NA	0.38±0.14
Aripo B	1,182 N, 694 E	Low ¹	R	Aripo ^C	27	1.74	0.07±0.05
Aripo I	1,180 N, 694 E	Low ²	R	Aripo ^C	10	3.7	0
Quare 6	1,182 N, 698 E	Low ²	R	Quare ^O	17	3.18	0.12±0.12
Quare 7	1,180 N, 698 E	Low ³	R	Quare ^O	20	0.98	0
Paria 4	1,189 N, 689.5 E	Low ¹	R+M	Paria ^P	53	NA	0.19±0.11
Arima	1,174 N, 690 E	High ¹	C+A+Ch	Caroni ^C	33	2.94	0.52±0.13
Aripo 10	1,178 N, 695 E	High ²	C+A+Ch	Aripo ^C	29	1.75	0.38±0.14
Aripo 6a	1,178 N, 695.5 E	High ²	C+A+Ch	Aripo ^C	14	1.25	0
Ceniza	1,177 N, 696 E	High ¹	C+A+Ch	Aripo ^C	32	1.91	0.22±0.12
Hollis	1,182.5 N, 698 E	High ¹	C+A+Ch	Quare ^O	14	1.94	0.07±0.07
Manacal	1,171 N, 687 E	High ¹	C+A+Ch	Caroni ^C	16	2.55	0.38±0.26
Mausica A	1,173 N, 686 E	High ¹	C+A+Ch	Caroni ^C	71	2.90	2.76±0.42 ⁱ
Mausica B	1,172.5 N, 685 E	High ¹	C+A+Ch	Caroni ^C	20	2.40	0.20±0.09
Oropuche	1,179 N, 704 E	High ¹	C+A+Ch	Oropuche ^O	20	1.43	0.35±0.18
Oropuna A	1,172 N, 681 E	High ¹	C+A+Ch	Caroni ^C	39	1.17	0.67±0.19
Oropuna B	1,178 N, 683 E	High ¹	C+A+Ch	Caroni ^C	29	1.98	0.48±0.17
Turure	1,178.5 N, 700 E	High ¹	C+A+Ch	Quare ^O	20	2.55	0.10±0.07
Valencia	1,178 N, 696.5 E	High ¹	C+A+Ch	Aripo ^C	56	1.46	0.77±0.25 ^j

¹ Endler 1978; ² Reznick and Endler 1982; ³ Rodd et al. 2002

^aNames followed by numerals refer to Endler's original naming system. All other sites are new to this study and numbered with capital letters when necessary.

^bTrinidad National Grid System, 1:25,000 map series (1 cm=0.25 km)

^cReferences for predation intensity are provided.

^dThe most relevant aquatic predators of guppies are *Crenicichla alta* (C), *Aequidens pulchur* (A), several Characins (Ch), *Rivulus hartii* (R), and the prawn *Macrobrachium crenulatum* (M).

^eDrainages are part of the larger Caroni, Oropuche, or Paria river systems, denoted by a C, O, or P next to their name.

^fRefers to the number of male fish sampled for parasites

^gData not available (NA) for two populations

^h*Gyrodactylus* spp. parasites (mean±SE) per male (see Fig. 1)

ⁱ1.8±0.29 parasites per male after correcting for greater sampling effort by averaging only the first 25 males sampled. This sample does not include the outlier male bearing 28 parasites

^j0.8±0.41 parasites per male after correcting for greater sampling effort by averaging only the first 25 males sampled

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