

## Adding parasites to the guppy-predation story: insights from field surveys

Kiyoko M. Gotanda · Lari C. Delaire · Joost A. M. Raeymaekers · Felipe Pérez-Jvostov · Felipe Dargent · Paul Bentzen · Marilyn E. Scott · Gregor F. Fussmann · Andrew P. Hendry

Received: 21 March 2012 / Accepted: 18 September 2012 / Published online: 9 October 2012  
© Springer-Verlag Berlin Heidelberg 2012

**Abstract** Studies of phenotypic variation in nature often consider only a single potential selective agent. In such cases, it remains an open question as to whether variation attributed to that single measured agent might be influenced by some other unmeasured agent. Previous research has shown that phenotypic variation in the Trinidadian guppy (*Poecilia reticulata*) is strongly influenced by predation regime, and we here ask whether parasitism might represent an additional important selective agent shaping this variation. We performed a field survey of 26 natural guppy populations of known predation regime in northern Trinidad. We quantified levels of parasitism of guppies by the

monogenean ectoparasite, *Gyrodactylus*, and examined whether this parasite was associated with guppy body size or male colour. Spatial variation in *Gyrodactylus* parasitism was consistent between years, and parasite prevalence was generally, but not always, higher at high-predation sites than at low-predation sites. Consistent with previous work, predation regime was related to guppy size and some aspects of male colour, whereas parasitism showed few and only minor associations with the same traits. Moreover, a consideration of parasitism did not alter any interpretations regarding associations between guppy traits and predation regimes. These results suggest that parasitism, at least as quantified in the present study, does not play a major role in shaping variation in guppy body size or colour. Nevertheless, considerable variation in these traits, even within a predation regime, suggests the likely importance of other selective agents beyond just predation regime.

Communicated by David Marcogliese.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00442-012-2485-7) contains supplementary material, which is available to authorized users.

K. M. Gotanda · L. C. Delaire · F. Pérez-Jvostov · F. Dargent · G. F. Fussmann · A. P. Hendry  
Department of Biology, McGill University, Montreal, QC H3A 1B1, Canada

K. M. Gotanda (✉) · L. C. Delaire · A. P. Hendry  
Redpath Museum, McGill University,  
859 Sherbrooke Street West, Montreal, QC H3A 0C4, Canada  
e-mail: kiyoko.gotanda@mail.mcgill.ca

J. A. M. Raeymaekers  
Laboratory of Biodiversity and Evolutionary Genomics,  
Katholieke Universiteit Leuven, 3000 Leuven, Belgium

F. Pérez-Jvostov · M. E. Scott  
Institute of Parasitology, Macdonald Campus of McGill  
University, Ste-Anne de Bellevue, QC H9X 3V9, Canada

P. Bentzen  
Department of Biology, Dalhousie University,  
Halifax, NS B3H 4R2, Canada

**Keywords** Adaptive divergence · Natural selection · *Poecilia reticulata* · Selective agents · Sexual selection

### Introduction

Studies of adaptation are often conceptualized in terms of unifactorial causality; that is, they focus on a single potential selective agent. In aquatic organisms, examples of selective agents that have figured heavily in unifactorial studies include predation (Endler 1980; Reznick and Endler 1982; Langerhans et al. 2004), dissolved oxygen (Mandic et al. 2009; Reardon and Chapman 2010), competition (Schluter 1994; Gray and Robinson 2002), and light intensity (Porter et al. 1984). Although these single selective agents often explain a considerable amount of the phenotypic variation among natural populations, most traits in most populations

are presumably under pressure from multiple selective agents. The question then becomes what is the relative importance of the different selective agents and how do they interact to shape phenotypic variation in nature.

An explicit consideration of multifactorial causality is important for several reasons. First, different selective agents could be correlated with each other to the extent that variation attributed to one (measured) agent could be instead driven by another (unmeasured) agent (MacColl 2011). For example, shell pigmentation and patterning in *Cepaea* (Held) was first attributed to the direct selective effect of predation by thrushes (Cain and Sheppard 1950), but it is now clear that other important ecological variables such as local vegetation can co-vary with predation intensity and influence the frequency distribution of shell phenotypes (Jones et al. 1977). Second, different causal agents might have interactive effects on phenotypes, which could obscure (if offsetting) or enhance (if complementary) the apparent effects of either agent considered alone. For example, Decaestecker et al. (2002) demonstrated that habitat selection by *Daphnia magna* (Straus) is subject to a trade-off between exposure to visual predators in shallow water and exposure to parasites in deep water. These are only a few examples highlighting the value of considering multifactorial causality when interpreting patterns of phenotypic variation in nature (MacColl 2011).

Here, we focus in more detail on multifactorial causality of phenotypic variation that might be related to predation and parasitism. Many studies have examined the potential influence of predation (e.g. Kerfoot and Sih 1987) and others are increasingly considering the role of parasites (Lozano 1994; Houde 1997; Lopez 1998; van Oosterhout et al. 2007; Vergara et al. 2011). Relatively few studies, however, have considered both—and such a consideration might be very important for two reasons. First, levels of predation and parasitism might be positively or negatively correlated, or might show more idiosyncratic patterns (Decaestecker et al. 2002; Dick et al. 2010; Johnson et al. 2010; Otti et al. 2012). For example, both selective agents can increase mortality rates, and so might jointly enhance the evolution of “fast” life histories (Roff 1992). Second, predators and parasites might select on the same traits in similar or different ways. As an example, predators might select for traits (e.g. habitat choice, development rate, or shoaling) that actually increase exposure to parasites (Hatcher et al. 2006; Raffel et al. 2010). All these observations suggest the value of exploring multifactorial causality in the context of predation and parasitism.

#### Our study system

Trinidadian guppy (*Poecilia reticulata*, Peters) populations are typically categorized into those experiencing high

predation (HP, strongly piscivorous fishes present) versus those experiencing low predation (LP, only weakly piscivorous fishes present). From a unifactorial perspective, this predation contrast explains a substantial portion of the variation in guppy morphology, behaviour, life history, and colour (Endler 1980, 1995; Reznick and Endler 1982; Strauss 1990; Houde 1997; Magurran 2005). For instance, LP guppies are usually larger and more brightly coloured than HP guppies (Endler 1980; Houde 1997; Magurran 2005). At the same time, however, guppy populations within a specific predation regime can often differ dramatically in these same traits (Endler 1978; Reznick et al. 1996; Weese et al. 2010; Millar and Hendry 2012), suggesting that other selective agents are also at play (Endler 1995). Indeed, recent work has suggested that guppy phenotypic variation is also shaped by variation in resource levels (Kodric-Brown 1989; Grether et al. 2001; Reznick et al. 2001; Millar et al. 2006; Schwartz and Hendry 2010), sexual selection (Endler and Houde 1995; Houde 1997; Rodd et al. 2002; Schwartz and Hendry 2007), and parasitism (Houde and Torio 1992; Martin and Johnsen 2007).

Among these potential selective agents, parasitism represents a particularly interesting agent, and we here focus on the monogenean ectoparasite, *Gyrodactylus*. These ubiquitous parasitic worms of teleost fishes have a direct life cycle and are transferred horizontally through fish contact (Harris and Lyles 1992; Bakke et al. 2007). They are particularly appropriate for our study for several reasons. First, natural guppy populations vary considerably in their levels of infection by two species of *Gyrodactylus* (Harris and Lyles 1992; Martin and Johnsen 2007; van Oosterhout et al. 2007; Fraser and Neff 2010): *Gyrodactylus turnbulli* (Harris) or *Gyrodactylus bullatarudis* (Turnbull). Second, *Gyrodactylus* infections influence survival, growth rate, and reproductive success in the laboratory, experimental stream channels, and wild guppies (Scott and Anderson 1984; Cable and van Oosterhout 2007b; van Oosterhout et al. 2007; Pérez-Jvostov et al. 2012), suggesting the potential for these parasites to impose selection. Third, laboratory studies have shown that infection by *Gyrodactylus* can influence a number of guppy traits that also differ between HP and LP populations, including foraging (Kolluru et al. 2009), shoaling (Johnson et al. 2011), male courtship behaviour (Kennedy et al. 1987; Lopez 1998), body size (Cable and Van Oosterhout 2007a), and carotenoid colouration (Houde and Torio 1992; Houde 1997), and individual-based modelling has shown a possible effect on body size (van Oosterhout et al. 2008).

The question we seek to answer is whether or not parasitism by *Gyrodactylus* leaves a signature on guppy phenotypes in natural populations. Our motivation for asking this specific question is that such effects are seemingly

strong for predation (Endler 1980; Reznick and Endler 1982; Magurran 2005) and resource levels (Kodric-Brown 1989; Grether et al. 2001; Reznick et al. 2001; Millar et al. 2006; Schwartz and Hendry 2010). These strong correlations in nature argue that the effects of those selective agents are so large as to emerge above all the noise of other potential agents shaping phenotypic variation in nature. Should parasitism be elevated to a similar level of importance? If so, we would expect field surveys to show strong associations between parasitism and guppy traits within and among natural guppy populations. Several relevant surveys have been previously conducted. For example, Fraser and Neff (2010) showed that, in natural populations, specific guppy genotypes were more likely to have higher *Gyrodactylus* infections. A mark–recapture study in one river system found parasitised males were more likely to be removed from a population (van Oosterhout et al. 2007), and Martin and Johnsen (2007) failed to find any association between parasite levels and orange colouration in male guppies. We expand on previous work by conducting a larger field survey with improved methods that also examines a larger suite of phenotypic traits.

We first assess variation in parasitism among natural guppy populations, asking whether it is consistent between years and whether it differs between predation regimes (HP vs. LP). We then test for associations between various indices of parasitism and a number of guppy traits at both the individual and population levels. Any such associations could be singly or jointly due to the effect of genetic adaptation and plasticity, given that most guppy traits are influenced by both (Kodric-Brown 1989; Houde 1992; Hughes et al. 2005; Ghalambor et al. 2007; Tripathi et al. 2009). Finally, we ask whether any interpretations regarding the effects of predation on guppy phenotypes are modified by a simultaneous consideration of parasitism.

## Materials and methods

### Field sampling and parasite assessment

In February 2009 and 2010, during the dry season, we sampled approximately 30 adult male and 30 adult female guppies from each of 26 sites across 10 rivers in northern Trinidad (Online Resources 1–2), most sites used in our previous work on guppy traits (Millar et al. 2006; Millar and Hendry 2012). The guppies at these sites are considered “populations” because the sites are far enough apart that gene flow between them is expected to be low (Crispo et al. 2006), at least in relation to the scale of adaptation (Endler 1995). Each site was categorised as either “high predation” (HP) or “low predation” (LP) based on previously described predator assemblages, which we confirmed

on our repeated visits. This binary HP versus LP categorization is traditional in the guppy literature, and reflects the presence versus absence of predatory fishes known to have major effects on guppy demographics (e.g. Reznick et al. 1996, 2001; Rodd et al. 2002; Millar et al. 2006; Martin and Johnsen 2007; van Oosterhout et al. 2007). At least one HP and one LP site was sampled within each of nine rivers (Online Resources 1–2). The tenth river (Paria) did not have an HP population large enough to sample.

Guppies were caught using butterfly nets, briefly placed into a bucket, immediately transferred to individual 18 oz Whirl–pak© bags (Fisher Canada) containing river water, and placed in an insulated cooler in the shade. This procedure quickly isolated each guppy to prevent parasites from moving between fish after capture. The fish were then transported to our laboratory in Trinidad and processed individually within 5 h of sampling. Each fish was euthanized in 0.02 % Tricaine Methanesulfonate (Finquel MS-222; Argent Laboratories, Redmond, WA, USA) buffered to a neutral pH with NaHCO<sub>3</sub>. The fish was then immediately assessed for sexual maturity, and visually scanned using a stereomicroscope to count all *Gyrodactylus* on the skin and fins. The two *Gyrodactylus* species cannot be distinguished under a stereomicroscope while on the skin (Harris 1986; Cable and Van Oosterhout 2007a) and are therefore considered together in our study as has been done in previous studies (Cable and Van Oosterhout 2007a; Fraser and Neff 2010; Fraser et al. 2010). The fish was then placed on a white background and its left side was photographed with a digital SLR (Nikon D80) equipped with a 60-mm macro lens and remote shutter. For these photos, the tail of the fish was carefully spread to its maximum extent with a fine paint brush. A ruler and a colour standard were included in each photograph, and illumination was provided by two full spectrum fluorescent lights and a Nikon Speedlight Commander Kit R1C1 flash.

We calculated several indices of parasitism in both 2009 and 2010. At the individual guppy level, parasitism was described by the categorical binary variable *infection presence* (infected or not) and by the discrete variable *parasite abundance* (the total number of *Gyrodactylus* per fish). As results were generally the same for the two variables, we focus primarily on the former in the presentation and discussion of our findings. At the population level, parasitism was described by *prevalence* (percentage of infected fish) and by *mean abundance* (average number of *Gyrodactylus* per fish, including uninfected fish).

### Guppy traits

Guppy traits were quantified from the photographs of 760 male and 831 female fish sampled only in 2009, given that the measurements are very time consuming and differences

in guppy traits among populations are consistent between years in the absence of major disturbances (Schwartz and Hendry 2010; Gotanda and Hendry, unpublished data). However, temporal stability in parasite levels on guppies has not often been examined in the field and we also wanted to determine if generalised characterisation of sites with regard to infection status was possible, so we here analyse 2 years of parasite data. All measurements were made by a single person (L.C.D.) who was blind to a fish's site of origin and infection level. The measurements fell into three basic categories: morphometrics for both males and females, "traditional" colour metrics for males, and colour spot properties for males.

Five morphometric measurements (Online Resource 3) were made on all fish (body length, tail length, body depth, body area and tail area) using the program Image J (<http://imagej.nih.gov/ij/>). Traditional colour metrics of male fish were based on the visual categorisation of individual colour spots on the body and tail into one of eight categories (modified from Endler 1991; Millar et al. 2006; Kemp et al. 2008): black, fuzzy black, orange (including red), yellow, blue (including purple), green, violet-blue, and silver. For each male, the number of spots of a given colour category was counted (spot number) and the area of each individual spot was assessed in Image J. For each fish, the areas of all spots for a given colour category were then summed to yield the total area of that colour. These total areas were then divided by the total fish area (body area + tail area), yielding the "relative area" of each colour.

In addition to traditional colour metrics, further insight into colour variation can be gained from information about spectral properties of colour, such as hue, saturation, and brightness (Endler 1990). The ideal approach would be to use spectrophotometry and visual modelling, as has been applied to guppies in a few studies (e.g. Kemp et al. 2008). However, this procedure is very time consuming, and was therefore incompatible with our need to obtain information from large numbers of fish sampled in a single day. We therefore instead quantified colour properties from photographs using an imaging program (Photoshop CS4, v.11.0.1). This is a procedure that has yielded useful insights and has previously been applied to guppies (Karino and Haijima 2001; Martin and Johnsen 2007). For details on quantification of hue, saturation and brightness, see Online Resource 3.

### Statistical analysis

Data analyses were conducted in the R environment (v.2.13.1.2011; R Development Core Team, 2011). To evaluate variation in parasitism, we first examined inter-annual consistency of among-site differences by calculating the correlation (Pearson's  $r$ ) between 2009 and 2010 for the

population-level parasite *prevalence* and *mean abundance*. Due to logistical constraints, the Quare LP site was not sampled in 2010 and was thus excluded from this particular analysis. We then tested for factors associated with variation in parasitism. At the individual level, a generalised linear mixed model (GLMM) with binomial errors was used to test whether *infection presence* was related to the fixed factors predation regime, year, sex, and their interactions, as well as site nested within predation regime as a random factor. A GLMM with negative binomial errors and log link function was used to test if *parasite abundance* was related to the same factors. The three-way interaction was non-significant in the first model (*infection presence*), and did not influence any of the results, and so it was removed from this model. At the population level, general linear models (GLMs) were used to test whether parasite *prevalence* or *mean abundance* were related to the fixed effects of year, predation regime, and their interaction.

We next tested for associations between parasitism and guppy traits by means of individual-level analyses (data points were individual guppies) and population-level analyses (data points were mean values for all guppies at a given site). Individual-level analyses were used to infer whether trait values differed between parasitised and unparasitised individuals within populations (responses of individuals to being parasitised) and among populations (particularly with regard to predation regime). Population-level analyses were then conducted on the assumption that individual-level effects might influence trait divergence between populations. All tests hence forth used a Type I Sequential SS, and we conducted all analyses with predation fitted before parasitism and with parasitism fitted before predation. For brevity, we do not report results of the latter because changing the order had minimal effect on the outcome.

At the individual level, MANOVAs with individual male guppy traits as response variables were used to test for fixed effects of predation regime, parasitism (*infection presence* or *parasite abundance* in separate models), their interaction, and site nested within predation regime. Site was here treated as a fixed factor, which is required in MANOVA. At the population level, MANOVAs with the population averages of guppy traits as response variables were used to test for effects of predation regime, parasite *prevalence* or *mean abundance* (in separate models), and their interactions. These analyses were implemented for two different sets of response variables: the five body size measurements, and the colour properties (HSB) of black, orange, yellow and green spots. The third set of response variables ("traditional" colour metrics) were analysed in similar models; they differed only in that body size (PC1 based on the five morphometric measurements) was added as a covariate (i.e. MANCOVA). Separate models were run for the numbers of spots and for the relative areas of colour, and analyses based

on the average size of individual spots are not reported here as no new information was gained.

The above statistical models were supplemented by additional analyses intended to aid specific inferences. First, we wished to infer whether interpretations regarding predation were altered by the simultaneous consideration of parasitism. We therefore performed all of the above analyses again but excluded the parasitism term, and then (1) determined whether the significance of the predation term was altered, and (2) used a likelihood ratio test to compare the fit of the models with and without parasitism. Second, we wished to determine whether statistical inferences based on MAN(C)OVAs depended on particular response variables, as opposed to their multivariate combination. We therefore also performed selected post hoc univariate AN(C)OVAs that considered individual response variables in models that had the same structure as the multivariate models. The only difference was that, unlike multivariate analyses, univariate analyses allow the nesting of random factors, and so “site” could now be treated as random.

## Results

### Patterns of parasitism

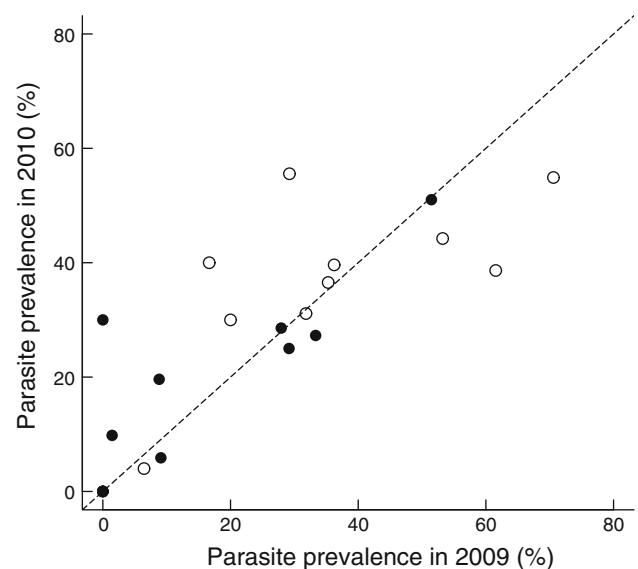
We first considered variation in parasitism within and among guppy populations. Among-population variation in parasitism was highly correlated between years when assessed as parasite prevalence (Fig. 1;  $r = 0.846$ ,  $P < 0.0001$ ) or mean abundance ( $r = 0.579$ ,  $P = 0.0024$ ). When considering predation regime and sex effects, analyses at the individual level (Table 1) revealed that infection presence (Fig. 2) and parasite abundance was generally higher at HP sites than at LP sites. A significant interaction between predation regime and sex revealed infection presence and parasite abundance was higher for females than for males at HP sites, but higher for males than for females at LP sites. Although not statistically significant, parasite prevalence appeared qualitatively higher overall in HP sites than in LP sites (Fig. 2), although exceptions were evident for specific rivers (Aripo, Marianne and Quare; Fig. 2). Neither predation regime nor year had a significant effect on mean abundance (Table 1).

### Effects on guppy traits

We next considered variation in guppy morphometric traits. Individual-level and population-level multivariate analyses revealed that LP fish were larger than HP fish, and individual-level analysis also revealed that females were larger than males (Table 2; Fig. 3). At the individual level, significant effects were also found for site, infection

presence (parasitised fish were larger than unparasitised fish) or parasite abundance (larger fish had more parasites), the interaction between sex and predation (the difference in size between predation regimes was greater for females), and the three-way interaction between sex, parasitism, and predation (Table 2). For infection presence, the interaction between predation and parasitism indicated that the difference in guppy size between predation regimes was greater for uninfected fish. For parasite abundance, the interaction between predation and parasitism indicated that the effect of fish size on the number of parasites was greater in HP sites than in LP sites. Post hoc ANOVAs showed that predation and sex were strong predictors of all or most size metrics, whereas the significant effect of infection presence applied only to body area and body length and parasite abundance did not have a significant effect on any size metric (Online Resource 4). At the population level, neither prevalence nor mean abundance had an effect on body size (Table 2).

With respect to “traditional” colour metrics, population-level multivariate analyses did not reveal any significant effects of predation nor parasitism on the number of coloured spots on males (Online Resource 5). At the individual level, for brevity, we only report the results of infection presence as the results with parasite abundance as an independent variable were comparable. Individual-level multivariate analyses, revealed significant effects of



**Fig. 1** Parasite (*Gyrodactylus*) prevalence of Trinidadian guppy (*Poecilia reticulata*) populations at low (filled circles) and high (open circles) predation sites in 2009 versus 2010 ( $n = 25$ ). The dashed line represents equal parasitism in both years (1:1). Quare LP is not included due to logistical problems related to sampling in 2010. Seven LP sites had no infected fish in either year (Online Resource 2) and are all represented together as a single data point in the lower left-hand corner

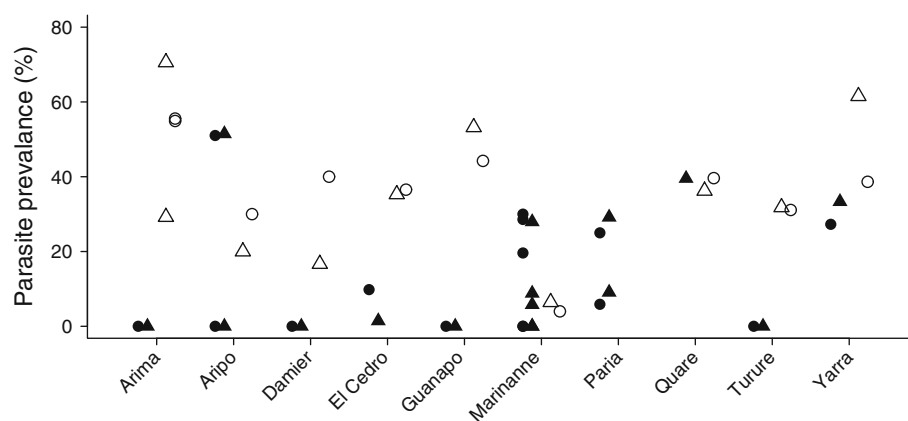
**Table 1** Parasitism as the response variable in the GLMM and GLMs examined in relation to predation, year, sex, and their interactions at the individual level ( $n = 2,823$ ) and population level ( $n = 25$ ) level

Variable	Individual level— <i>infection presence</i>		Individual level— <i>parasite abundance</i>	
	<i>z</i>	<i>P</i>	<i>z</i>	<i>P</i>
Predation	−3.400	<b>0.0007</b>	−3.22	<b>0.0013</b>
Year	0.202	0.8402	1.68	0.0928
Sex	−3.400	<b>0.0018</b>	−2.35	<b>0.0190</b>
Predation × year	0.013	0.9897	0.53	0.5958
Predation × sex	2.693	<b>0.0071</b>	3.79	<b>0.0002</b>
Year × sex	1.762	0.0781	0.49	0.6211
Year × sex × predation			−1.72	0.0847

Variable	Population level— <i>prevalence</i>		Population level— <i>mean abundance</i>	
	<i>z</i>	<i>P</i>	<i>z</i>	<i>P</i>
Predation	−1.368	0.171	−1.590	0.112
Year	0.063	0.950	0.920	0.357
Predation × year	−0.004	0.997	−0.908	0.364

$P < 0.05$  are in bold type



**Fig. 2** Parasite prevalence of guppies in low (*solid symbols*) and high (*open symbols*) predation populations indicated by river for 2009 (*triangles*) and 2010 (*circles*). Each data point represents a site- and year-specific mean. No parasites were present in either year at 7 low-predation sites, indicated by *solid symbols* along the *x* axis. The

Arima, Marianne, and Paria rivers were sampled at multiple sites, and have more data points. The Paria river did not have a high-predation site large enough to sample, so all data points represent low-predation sites. Data points by year are slightly offset for clarity. Data for the Quare LP is for 2009 only

predation (LP fish had more spots than did HP fish) and site nested within predation regime (Online Resource 5). For relative colour area, population-level multivariate analyses did not reveal any significant effects (Online Resource 6). Individual-level analyses, however, revealed significant effects of predation (LP fish had more relative colour than HP fish), parasitism (uninfected fish had more relative colour than infected fish), the interaction between predation and parasitism (infected fish had more relative colour than uninfected fish in LP sites, whereas the reverse was true in HP sites), and site nested within predation regime (Online Resource 6). Post hoc ANCOVAs did not reveal significant effects of predation or parasitism on any of the individual

colours at the individual or population level (Online Resources 7–10), suggesting that no single colour was responsible for the above multivariate differences between predation regimes.

Multivariate analysis of hue, saturation, and brightness (HSB) at the population-level did not reveal any significant effects (Online Resource 11). Individual-level multivariate analyses, however, revealed significant effects of predation (LP sites tended to have higher HSB properties) and site nested within predation regime (Online Resource 11), and for the metric *parasite abundance*, a significant effect of parasitism (HSB and *parasite abundance* had a negative correlation). Post hoc ANOVAs did not reveal significant

**Table 2** Body morphometrics as the response variables in MANOVAs examined in relation to sex, predation regime, and parasitism at the individual and population levels

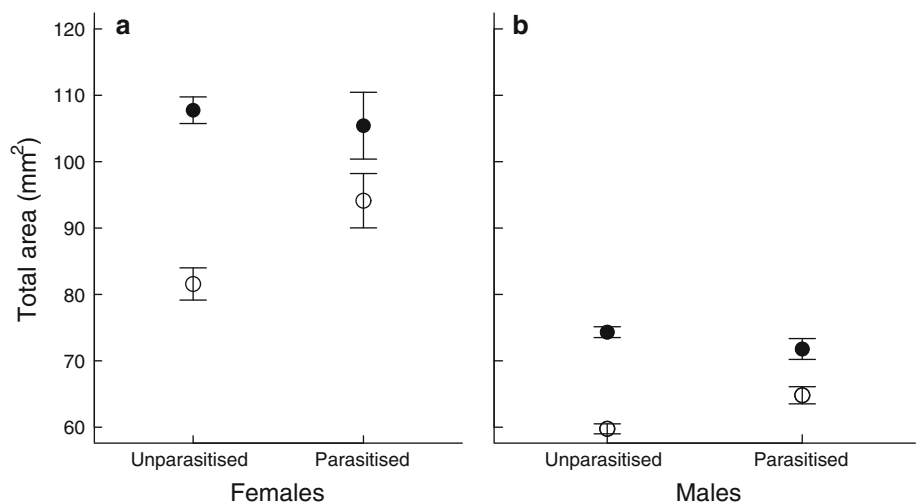
Variable	Individual level— <i>infection presence</i>			Individual level— <i>parasite abundance</i>		
	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
Predation	151.65	5, 1555	<b>&lt;0.0001</b>	151.55	5, 1555	<b>&lt;0.0001</b>
Parasitism	7.77	5, 1555	<b>&lt;0.0001</b>	4.12	5, 1555	<b>0.0010</b>
Sex	559.54	5, 1555	<b>&lt;0.0001</b>	557.44	5, 1555	<b>&lt;0.0001</b>
Pred × para	11.58	5, 1555	<b>&lt;0.0001</b>	11.47	5, 1555	<b>&lt;0.0001</b>
Sex × pred	3.82	5, 1555	<b>0.0019</b>	3.87	5, 1555	<b>0.0017</b>
Sex × para	1.39	5, 1555	0.2246	1.55	5, 1555	0.1701
Sex × pred × para	2.28	5, 1555	<b>0.0441</b>	0.76	5, 1555	0.5756
Site (predation)	29.54	120, 7647.3	<b>&lt;0.0001</b>	29.58	120, 7647.3	<b>&lt;0.0001</b>

Variable	Population level— <i>prevalence</i>		Population level— <i>mean abundance</i>	
	<i>F</i> <sub>5, 18</sub>	<i>P</i>	<i>F</i> <sub>5, 18</sub>	<i>P</i>
Predation	5.536	<b>0.0029</b>	5.474	<b>0.0031</b>
Parasitism	0.792	0.5692	0.747	0.5987
Pred × para	0.348	0.8769	0.4218	0.8275

*P* < 0.05 are in bold type

**Fig. 3** Total guppy (*Poecilia reticulata*) area (body + tail, mm<sup>2</sup>, mean ± SE) in for unparasitised and parasitised **a** females and **b** males from low (solid circles) or high (open circles) predation sites



effects of predation nor parasitism for any of the specific HSB measurements at the individual (*parasite abundance*) or population level (*parasite abundance*, Online Resource 12–13; Fig. 5), suggesting (as for traditional colour metrics) that the differences between predation regimes were not driven by any particular colour variable.

**Predation, parasitism, and guppy traits**

One of our main questions was whether a consideration of parasitism altered interpretations regarding predation. In this regard, we found that inclusion versus exclusion of parasitism from each of the above models did not change the significance of parameters associated with the effects of

predation for body size (Table 2; Online Resource 14), spot number (Online Resources 5 and 15), relative colour area (Online Resources 6 and 16), and colour spot properties (HSB; Online Resources 11 and 17). Furthermore, the inclusion versus exclusion of parasitism did not significantly alter the fit of any of the models to the data (Online Resource 18).

**Discussion**

**Patterns of parasitism**

If a particular selective agent is to shape spatial variation in phenotypic traits, it should differ strongly among

populations in a manner that is reasonably consistent through time. This is certainly the case for the predation regime (HP or LP) experienced by guppies (Endler 1980; Houde 1997; Reznick et al. 2001; Magurran 2005), and we first asked whether the same was true for infection by the monogenean ectoparasite, *Gyrodactylus*. We found inter-population differences in *parasite prevalence* (proportion of guppies that were infected) and *abundance* (average number of parasites on the sampled fish in a population) that were large and consistent between years. For instance, some LP populations in the Arima, Aripo, Damier, Guanapo Marianne, and Ture rivers showed no evidence of infection in either year, whereas up to half the fish in HP populations in the Arima, Guanapo, and Yarra and in an LP population in the Aripo were infected in both years. In addition, these results were consistent with previous surveys by other authors. For instance, Fraser and Neff (2010) also reported the absence of *Gyrodactylus* in LP populations of Guanapo and Ture, Fraser et al. (2010) found no difference between parasite prevalence nor abundance between 2 years of sampling within populations, and Cable and Van Oosterhout (2007b) found a *Gyrodactylus* prevalence of up to 50 % in the same LP Aripo population sampled in the present study.

Given the appearance of annual, temporally-consistent spatial variation in parasitism, its selective influence on guppies should depend on covariance with other specific agents, particularly predation regime. Here, we found that parasite *prevalence* was usually higher in HP populations as compared to LP populations (Table 1; Fig. 3), a result consistent with the single-year survey of Martin and Johnsen (2007), and the 2-year survey of Fraser et al. (2010). The specific reason for this association is not yet known, but a number of hypotheses can be advanced, and we suggest a few. First, infected fish tend to be displaced downstream during high water flow events such as flooding (van Oosterhout et al. 2007), and so LP sites above waterfalls might differentially lose infected fish to HP sites below waterfalls. Flooding can occur during both the wet and dry seasons due to flash floods associated with severe thunderstorms. Second, guppies in HP populations often shoal more than do guppies in LP populations (Magurran 1990, 2005), which might increase parasite transmission in the former and thereby maintain higher parasite levels (Barber et al. 2000; Rifkin et al. 2012). Third, susceptibility to parasites might be higher in HP populations than in LP populations, but we found no evidence of this in a recent mesocosm study (Pérez-Jvostov et al. 2012), though the contrary has been found in a previous study of one population (van Oosterhout et al. 2006). Fourth, downstream sites might also be warmer and have high conductivity (Reznick et al. 2001), which could contribute to higher parasitism rates (Scott and Nokes 1984; Bagge et al. 2004).

Given that predation regime and parasite levels were associated with each other, our ability to tease apart their relative effects on guppy traits depended on their association not being too strong. We found this, as some HP populations had lower parasite *prevalence* and *abundance* than some LP populations, even within the same river (e.g. Aripo and Marianne). Interestingly, these exceptions to the general rule were consistent between years in our study, including the relatively high *infection prevalence* in the Aripo LP population surveyed by van Oosterhout et al. (2007). This consistency suggest that predation regime per se is not the only selective agent causing variation in parasite levels but rather some other agents that are sometimes associated with predation regime. What agents these might be are not yet known. Regardless, the imperfect association between predation and parasitism provides optimism that their separate effects on guppy traits, if present and strong, should be detectable in a large field survey such as ours.

#### Parasitism and guppy traits

Our results suggest that *Gyrodactylus* is not an important selective agent shaping natural variation in guppy body size and colour. Infected fish were larger within HP populations, possibly because they are a larger target for parasites, because larger fish can better withstand parasitism, or because larger fish are more likely to reproduce and the stress of reproduction might lower immune-defence. However, body size was not associated with parasitism (*prevalence* or *mean abundance*) across populations. For colour, infected and uninfected males differed only in relative colour area (uninfected fish had marginally more colour) within populations, and parasitism was not associated with male colour variables across populations except perhaps a small (multivariate only) effect of *parasite abundance* on HSB properties. These results are generally consistent with Martin and Johnsen (2007), who did not find any association between *Gyrodactylus* parasitism and orange colour across Trinidadian guppy populations. These negative results might seem surprising given the many reasons why male guppy traits might show associations with parasitism (see “Introduction”).

We can see several possible reasons why *Gyrodactylus* infection might influence guppy traits in the laboratory without such effects being detectable in surveys of natural populations. First, the static nature of point-in-time field sampling does not account for an individual guppy's infection history or the history of recent epidemics in the population. This is a concern because laboratory and mesocosm studies have found that *Gyrodactylus* epidemics can spread rapidly and then decline both on individual guppies and within guppy populations (Scott and Anderson 1984; Scott 1985, 1987; Pérez-Jvostov et al. 2012). This



variation in parasite abundance means that the measured level of parasitism at one time will not necessarily reflect the levels present during the phase of parasite population growth on an individual. Second, laboratory studies control for variation in other selective agents that might influence guppy traits, such as resource acquisition (Grether et al. 1999, 2001; Kolluru et al. 2009), infection by other parasites (Kennedy and Guégan 1994), and guppy age (Thomas et al. 2009). Perhaps these other agents mask any effects of parasitism that might occur in natural populations. Third, differential mortality of infected versus uninfected guppies (Scott and Anderson 1984; Gheorgiu et al. 2006; van Oosterhout et al. 2007) might alter apparent parasitism levels and guppy trait distributions in different ways in different populations. Discriminating among these—and other—hypotheses will require tracking individual guppies through time, or performing manipulative experiments in nature.

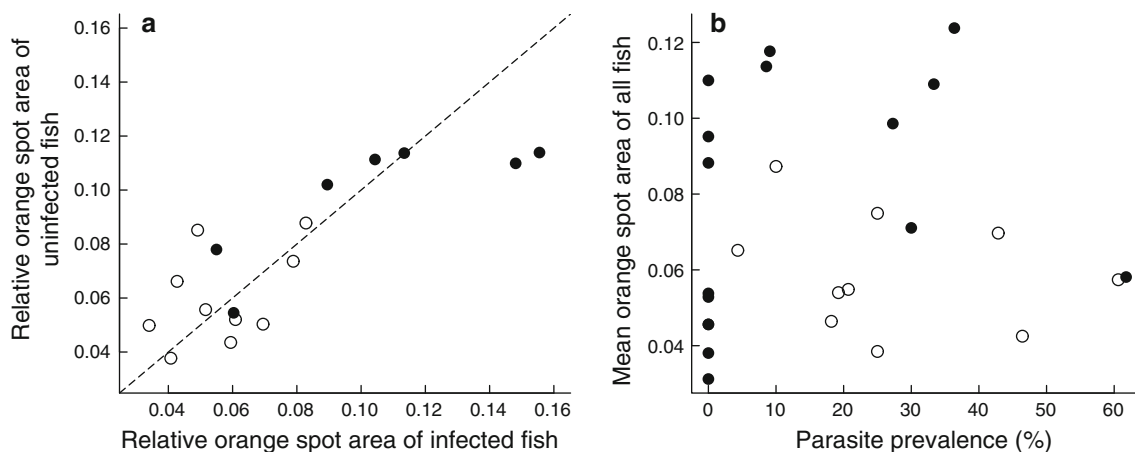
### Predation, parasitism, and guppy traits

Does information on parasitism modify interpretations regarding predation? We can first affirm that variation in some guppy traits is indeed associated with predation regime: most strikingly, guppies are larger in LP sites than in HP sites (Fig. 3). This pattern has been observed in previous work, and is due to a combination of plastic, demographic, and evolutionary responses to higher mortality rates and lower competition in HP sites than in LP sites (Endler 1980; Reznick and Endler 1982; Rodd and Reznick 1997; Grether et al. 2001). In addition, some LP guppy populations had more orange on their bodies (Fig. 4) and their colour spots were more saturated (Fig. 5). These results also generally fit with previous work asserting that predation regime influences guppy colour patterns, ostensibly because of spatial variation in the balance between

sexual selection for conspicuousness and predator-based natural selection for crypsis (Endler 1980; Millar et al. 2006; Kemp et al. 2008).

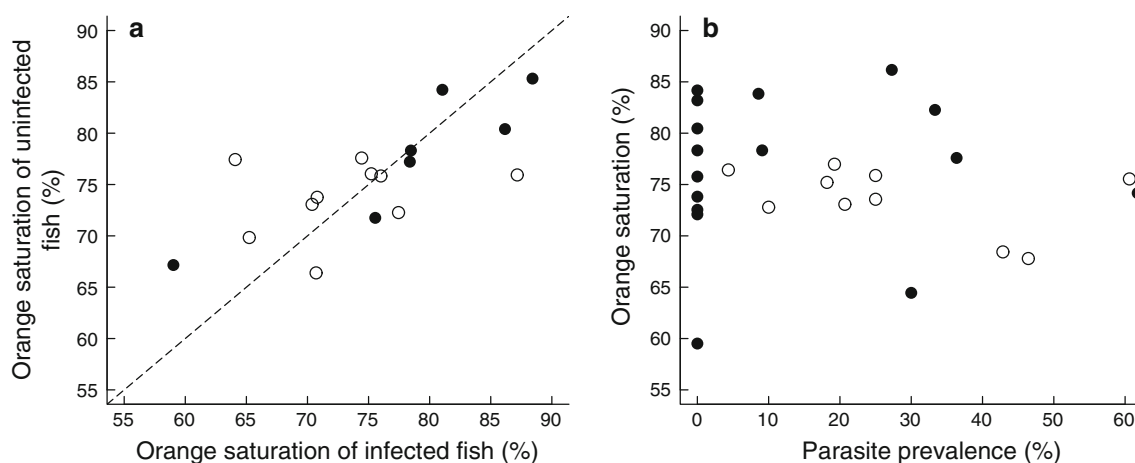
Despite this general confirmation of expectations regarding the effects of predation regime, the associations were often weak and inconsistent. In particular, predation regime was not a significant main effect at the population level for most colour variables. In addition, considerable overlap in colour variables was evident between predation regimes, even for orange (Fig. 4). For example, some LP guppy populations had males with less orange area and lower colour saturation than did some HP guppy populations (Figs. 4, 5). Similar complications and nuances have been revealed in other recent studies of the relationship between predation regime and guppy colour patterns (Millar et al. 2006; Kemp et al. 2008; Weese et al. 2010; Millar and Hendry 2012). Thus, while recent work suggests that predation regime is certainly important, the causes of guppy colour variation in nature are undoubtedly multifactorial.

The key question then becomes: does the predation regime story benefit from a simultaneous consideration of parasitism in the context of the role of selective agents? As noted above, our survey found that associations between *Gyrodactylus* parasitism and guppy traits were either absent or minor, and we would not therefore expect parasitism to have a serious modifying influence on interpretations regarding predation. Fitting this expectation, the inclusion versus exclusion of parasitism from statistical models had no influence on the apparent effects of predation and did not improve the fit of the models. Perhaps, then, we need not be overly concerned with *Gyrodactylus* parasites when interpreting the effects of predation regime on guppy body size and colour. Of course, this statement must be tempered by the above caveats regarding the inference of parasitism effects from field surveys.



**Fig. 4** The relative area of orange colour (area of orange colour divided by body area) on male guppies in relation to parasitism and predation regime. High-predation sites are represented by *open circles*

and low-predation sites by *solid circles*. Nine sites are not shown because no males were infected



**Fig. 5** The saturation (0–100 %) of orange spots on male guppies in relation to parasitism and predation regime. High-predation sites are represented by *open circles* and low-predation sites by *filled circles*. Nine sites are not shown in the graph because no males were infected

## Conclusions

Our main findings were that levels of *Gyrodactylus* parasitism (1) varied among guppy populations in a manner that was consistent across years, (2) was often (but not always) higher in high-predation regimes than in low-predation regimes, (3) was only rarely or weakly associated with guppy size or colour, and (4) did not modify interpretations regarding the effects of predation on guppy traits. These results seem to suggest that parasitism, at least as it was quantified in our study, is not important in shaping guppy phenotypic variation in nature. However, it is also possible that the point-in-time nature of sampling was not particularly effective at revealing such effects. In addition, other environmental agents in natural populations might mask or otherwise obscure any effects of *Gyrodactylus*. Also, other guppy traits, such as life history or behaviour, might be more strongly influenced by parasitism—and other types of parasites might prove to be more important. Thus, while we cannot say that parasitism is unimportant in shaping guppy trait variation, we certainly can say that any such effects are not detectable for the specific traits and parasites we studied in field surveys of natural populations. If such effects really are present, they are apparently swamped—more so than effects of predation regime—by other agents influencing guppy trait variation in nature. Much of the variation in guppy traits remains to be explained both within and among populations, suggesting that multiple causal agents—some yet to be discovered—are important in this system.

**Acknowledgments** Fieldwork was aided by Shahin Muttalib, Lyndsey Baillie, and Ian Paterson. Photoshop analysis was performed by Cameron Mojarrad. Special thanks to Indar Ramnarine for field support and Mauricio Torres for producing Online Resource 1. We sincerely thank three anonymous reviewers and David J. Marcogliese

for their comments which greatly improved the manuscript. Funding for field work was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) in the form of a Special Research Opportunity Grant to G.F.F., M.E.S., P.B., and A.P.H.. NSERC also provided a Canada Graduate Scholarship—Masters and Vanier Canada Graduate Scholarship to K.M.G. J.A.M.R. was funded by the Research Foundation—Flanders and the University of Leuven, F.D. was funded by a Richard H. Tomlinson Fellowship, and F.P.J. was funded by the Consejo Nacional de Ciencia y Tecnología (Mexico). Research at the Institute of Parasitology is supported by a regroupement stratégique from Fonds Québécois pour la Recherche sur la Nature et les Technologies (FQRNT).

## References

- Bagge AM, Poulin R, Valtonen ET (2004) Fish population size, and not density, as the determining factor of parasite infection: a case study. *Parasitology* 128:305–313
- Bakke TA, Cable J, Harris PD (2007) The biology of gyrodactylid monogeneans: the “Russian-doll killers”. In: Baker JR, Muller R, Rollinson D (eds) *Advances in parasitology*, vol 64. Academic press, New York, pp 161–460
- Barber I, Hoare D, Krause J (2000) Effects of parasites on fish behaviour: a review and evolutionary perspective. *Rev Fish Biol Fish* 10:131–165
- Cable J, Van Oosterhout C (2007a) The impact of parasites on the life history evolution of guppies (*Poecilia reticulata*): the effects of host size on parasite virulence. *Int J Parasitol* 37:1449–1458
- Cable J, van Oosterhout C (2007b) The role of innate and acquired resistance in two natural populations of guppies (*Poecilia reticulata*) infected with the ectoparasite *Gyrodactylus turnbulli*. *Biol J Linn Soc* 90:647–655
- Cain AJ, Sheppard PM (1950) Selection in the polymorphic land snail *Cepaea nemoralis*. *Heredity* 4:275–294
- Crispo E, Bentzen P, Reznick DN, Kinnison MT, Hendry AP (2006) The relative influence of natural selection and geography on gene flow in guppies. *Mol Ecol* 15:49–62
- Decaestecker E, de Meester L, Ebert D (2002) In deep trouble: habitat selection constrained by multiple enemies in zooplankton. *Proc Natl Acad Sci USA* 99:5481–5485
- Dick JTA et al (2010) Parasitism may enhance rather than reduce the predatory impact of an invader. *Biol Lett* 6:636–638

- Endler JA (1978) A predator's view of animal color patterns. *Evol Biol* 11:319–364
- Endler JA (1980) Natural selection on color patterns in *Poecilia reticulata*. *Evolution* 34:76–91
- Endler JA (1990) On the measurement and classification of color in studies of animal color patterns. *Biol J Linn Soc* 41:315–352
- Endler JA (1991) Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vis Res* 31:587–608
- Endler JA (1995) Multiple trait coevolution and environmental gradients in guppies. *Trends Ecol Evol* 10:22–29
- Endler JA, Houde AE (1995) Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* 49:456–468
- Fraser B, Neff B (2010) Parasite mediated homogenizing selection at the MHC in guppies. *Genetica* 138:273–278
- Fraser BA, Ramnarine IW, Neff BD (2010) Temporal variation at the MHC class IIB in wild populations of the guppy (*Poecilia reticulata*). *Evolution* 64:286–2096
- Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol* 21:394–407
- Gheorgiu C, Marcogliese DJ, Scott M (2006) Concentration-dependent effects of waterborne zinc on population dynamics of *Gyrodactylus turnbulli* (Monogenea) on isolated guppies (*Poecilia reticulata*). *Parasitology* 132:225–232
- Gray SM, Robinson BW (2002) Experimental evidence that competition between stickleback species favours adaptive character divergence. *Ecol Lett* 5:264–272
- Grether GF, Hudon J, Millie DF (1999) Carotenoid limitation of sexual coloration along an environmental gradient in guppies. *Proc R Soc Lond B* 266:1317–1322
- Grether GF, Millie DF, Bryant MJ, Reznick DN, Mayea W (2001) Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology* 82:1546–1559
- Harris PD (1986) Species of *Gyrodactylus* von Nordmann, 1832 (Monogenea Gyrodactylidae) from poeciliid fishes, with a description of *G. turnbulli* sp. nov. from the guppy, *Poecilia reticulata*. *Peters. J Nat Hist* 20:183–191
- Harris PD, Lyles AM (1992) Infections of *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli* on guppies (*Poecilia reticulata*) in Trinidad. *J Parasitol* 78:912–914
- Hatcher MJ, Dick JTA, Dunn AM (2006) How parasites affect interactions between competitors and predators. *Ecol Lett* 9:1253–1271
- Houde AE (1992) Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poeciliidae) (guppies). *Heredity* 69:229–235
- Houde AE (1997) Sex, color, and mate choice in guppies. Princeton University Press, Princeton
- Houde AE, Torio AJ (1992) Effect of parasitic infection on male color pattern and female choice in guppies. *Behav Ecol* 3:346–351
- Hughes KA, Rodd FH, Reznick DN (2005) Genetic and environmental effects on secondary sex traits in guppies (*Poecilia reticulata*). *J Evol Biol* 18:35–45
- Johnson PTJ et al (2010) When parasites become prey: ecological and epidemiological significance of eating parasites. *Trends Ecol Evol* 25:362–371
- Johnson MB, Lafferty KD, van Oosterhout C, Cable J (2011) Parasite transmission in social interacting hosts: Monogenean epidemics in guppies. *PLoS ONE* 6:e22634
- Jones JS, Leith BH, Rawlings P (1977) Polymorphism in *Cepaea*: a problem with too many solutions? *Annu Rev Ecol Syst* 8:109–143
- Karino K, Haijima Y (2001) Heritability of male secondary sexual traits in feral guppies in Japan. *J Ethol* 19:33–37
- Kemp DJ, Reznick DN, Grether GF (2008) Ornamental evolution in Trinidadian guppies (*Poecilia reticulata*): insights from sensory processing-based analyses of entire colour patterns. *Biol J Linn Soc* 95:734–747
- Kennedy CR, Guégan JF (1994) Regional versus local helminth parasite richness in British freshwater fish—saturated or unsaturated parasite communities. *Parasitology* 109:175–185
- Kennedy CEJ, Endler JA, Poynton SL, McMinn H (1987) Parasite load predicts mate choice in guppies. *Behav Ecol Sociobiol* 21:291–295
- Kerfoot WC, Sih A (1987) Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover
- Kodric-Brown A (1989) Dietary carotenoids and male mating success in the guppy—an environmental component to female choice. *Behav Ecol Sociobiol* 25:393–401
- Kolluru GR, Grether GF, Dunlop E, South SH (2009) Food availability and parasite infection influence mating tactics in guppies (*Poecilia reticulata*). *Behav Ecol* 20:131–137
- Langerhans RB, Layman CA, Shokrollahi AM, DeWitt TJ (2004) Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution* 58:2305–2318
- Lopez S (1998) Acquired resistance affects male sexual display and female choice in guppies in Trinidad. *Proc R Soc Lond B* 265:717–723
- Lozano GA (1994) Carotenoids, parasites, and sexual selection. *Oikos* 70:309–311
- MacColl ADC (2011) The ecological causes of evolution. *Trends Ecol Evol* 26:514–522
- Magurran AE (1990) The adaptive significance of schooling as an anti-predator defence in fish. *Ann Zool Fenn* 27:51–66
- Magurran AE (2005) Evolutionary ecology: the Trinidadian guppy. Oxford University Press, New York
- Mandic M, Todgham AE, Richards JG (2009) Mechanisms and evolution of hypoxia tolerance in fish. *Proc R Soc Lond B* 276:735–744
- Martin CH, Johnsen S (2007) A field test of the Hamilton-Zuk hypothesis in the Trinidadian guppy (*Poecilia reticulata*). *Behav Ecol Sociobiol* 61:1897–1909
- Millar N, Hendry A (2012) Population divergence of private and non-private signals in wild guppies. *Environ Biol Fish* 513–525
- Millar NP, Reznick DN, Kinnison MT, Hendry AP (2006) Disentangling the selective factors that act on male colour in wild guppies. *Oikos* 113:1–12
- Otti O, Gantenbein-Ritter I, Jacot A, Brinkhof MWG (2012) Immune response increases predation risk. *Evolution* 66:732–739
- Pérez-Jostov F, Hendry AP, Fussmann GF, Scott ME (2012) Are host-parasite interactions influenced by adaptation to predators? A test with guppies and *Gyrodactylus* in experimental stream channels. *Oecologia* 170:77–88
- Porter JW, Muscatine L, Dubinsky Z, Falkowski PG (1984) Primary production and photoadaptation in light- and shade-adapted colonies of the symbiotic coral, *Stylophora pistillata*. *Proc R Soc Lond B* 222:161–180
- Raffel TR, Hoverman JT, Halstead NT, Michel PJ, Rohr JR (2010) Parasitism in a community context: trait-mediated interactions with competition and predation. *Ecology* 91:1900–1907
- Reardon EE, Chapman LJ (2010) Hypoxia and energetics of mouth brooding: is parental care a costly affair? *Comp Biochem Physiol A Comp Physiol* 156:400–406
- Reznick D, Endler JA (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* 36:160–177
- Reznick DN, Butler MJ, Rodd FH, Ross P (1996) Life-history evolution in guppies (*Poecilia reticulata*). VI. Differential

- mortality as a mechanism for natural selection. *Evolution* 50:1651–1660
- Reznick D, Butler MJ IV, Rodd FH (2001) Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *Am Nat* 157:126–140
- Rifkin JL, Nunn CL, Garamszegi LZ (2012) Do animals living in larger groups experience greater parasitism? A meta-analysis. *Am Nat* 180:70–82
- Rodd FH, Reznick DN (1997) Variation in the demography of guppy populations: the importance of predation and life histories. *Ecology* 78:405–418
- Rodd FH, Hughes KA, Grether GF, Baril CT (2002) A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proc R Soc Lond B* 269:475–481
- Roff DA (1992) The evolution of life histories: theory and analysis. Chapman and Hall, New York
- Schluter D (1994) Experimental evidence that competition promotes divergence in adaptive radiation. *Science* 266:798–801
- Schwartz AK, Hendry AP (2007) A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies (*Poecilia reticulata*). *Evol Ecol Res* 9:71–90
- Schwartz AK, Hendry AP (2010) Testing the influence of local forest canopy clearing on phenotypic variation in Trinidadian guppies. *Funct Ecol* 24:354–364
- Scott ME (1985) Dynamics of challenge infections of *Gyrodactylus bullatarudis* Turnbull (Monogenea) on guppies, *Poecilia reticulata* (Peters). *J Fish Dis* 8:495–503
- Scott ME (1987) Temporal changes in aggregation: a laboratory study. *Parasitology* 94:583–595
- Scott ME, Anderson RM (1984) The population dynamics of *Gyrodactylus bullatarudis* within laboratory populations of the fish host *Poecilia reticulata*. *Parasitology* 89:159–195
- Scott ME, Nokes DJ (1984) Temperature-dependent reproduction and survival of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). *Parasitology* 89:221–229
- Strauss RE (1990) Predation and life-history variation in *Poecilia reticulata* (Cyprinodontiformes: Poeciliidae). *Environ Biol Fish* 27:121–130
- Thomas F, Renaud F, Meeüs TD, Cézilly F, Cézilly F (2009) Parasites, age and the Hamilton-Zuk hypothesis: inferential fallacy? *Oikos* 74:305–309
- Tripathi N, Hoffmann M, Willing EM, Lanz C, Weigel D, Dreyer C (2009) Genetic linkage map of the guppy, *Poecilia reticulata*, and quantitative trait loci analysis of male size and colour variation. *Proc R Soc Lond B* 276:2195–2208
- van Oosterhout C et al (2006) Balancing selection, random genetic drift, and genetic variation at the major histocompatibility complex in two wild populations of guppies (*Poecilia reticulata*). *Evolution* 60:2562–2574
- van Oosterhout C et al (2007) Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *Int J Parasitol* 37:805–812
- van Oosterhout C, Potter R, Wright H, Cable J (2008) Gyro-scope: an individual-based computer model to forecast gyrodactylid infections on fish hosts. *Int J Parasitol* 38:541–548
- Vergara P, Martinez-Padilla J, Redpath SM, Mougeot F (2011) The ornament-condition relationship varies with parasite abundance at population level in a female bird. *Naturwissenschaften* 98:897–902
- Weese DJ, Gordon SP, Hendry AP, Kinnison MT (2010) Spatiotemporal variation in linear natural selection on body color in wild guppies (*Poecilia reticulata*). *Evolution* 64:1802–1815