Experimental Assessment in Nature of the Ecological Effects of a Specialist Parasite

Felipe Pérez-Jvostov[1], Andrew P. Hendry[1,2], Gregor F. Fussmann[1], and Marilyn E. Scott[3]

Parasites can have important effects on the structure and composition of natural biological communities, either directly by influencing host fecundity and survival (i.e., density-mediated effects) or indirectly by influencing host traits such as behavior, life-history, morphology, and physiology (i.e., trait-mediated effects). Yet few studies have explored how these effects play out *in the wild*, as opposed to simplified and controlled laboratory or mesocosm settings. We addressed this information gap by translocating *Gyrodactylus* ectoparasites of the Trinidadian guppy into previously *Gyrodactylus*-free guppy populations in two rivers (Marianne and Paria) in the Northern Mountain Range in Trinidad. We then measured phenotypic and demographic changes in the guppy host and its competitor, *Rivulus hartii*, and compared these changes to guppies and *Rivulus* in control reaches just upstream. In the Marianne, where guppies invest more in reproduction (i.e., greater size and number of embryos), the introduction of *Gyrodactylus* decreased guppy survival but did not influence guppy density or phenotypes. In the Paria, where guppies invest less in reproduction, the introduction of *Gyrodactylus* reduced female growth but did not influence guppy survival or density. In neither river did the introduction of *Gyrodactylus* influence the phenotype or demography of *Rivulus*. These results indicate some density-mediated and trait-mediated effects of parasites on hosts in natural setting, but also that these effects were context-specific, were generally weak, and did not cascade to a competitor. Give these outcomes, and their difference from typical lab-based studies, it is clear that more studies are needed that experimentally manipulate parasites in natural settings.

PARASITES are ubiquitous components of natural communities and ecosystems. Under this constant threat, hosts typically evolve defense mechanisms that prevent or reduce the impact of parasitic infections (Hedrick, 2004). This evolution can be manifested as increased resistance (ability to limit parasite infections) and/or tolerance (ability to limit the cost of a given infection level; Hedrick, 2004). However, the evolution of increased host defense then should lead to the evolution of increased parasite infectivity or virulence, further modifying the host-parasite interaction and likely influencing the population dynamics of both players (Hatcher et al., 2006).

Myriad studies have shown that parasites are important components of food webs (Marcogliese and Cone, 1997; Pedersen and Fenton, 2007; Johnson et al., 2010; Raffel et al., 2010) and hence influence ecosystem functioning (Hudson et al., 2006; Lafferty et al., 2006), biological invasions (Prenter et al., 2004; Dunn et al., 2012), and animal behavior (Barber and Dingemanse, 2010). These linkages suggest that the eco-coevolutionary interplay between hosts and parasites should have collateral influences on other members of the ecological community. For instance, infected hosts often show lethargic and erratic behaviors that can reduce their competitive ability (Hatcher et al., 2006) and/or increase their likelihood of being consumed by predators (Marcogliese, 2004). The resulting influence on competitor/predator growth and survival, and hence population dynamics (Minchella and Scott, 1993; Hatcher et al., 2006), then should have cascading influences on local biodiversity and ecosystem processes, such as primary productivity, nutrient cycling, and decomposition (Hatcher et al., 2012).

The general mechanisms by which parasites can influence hosts, and hence have further consequences for eco-evolutionary dynamics, can be categorized as density mediated (e.g., changes in host survival) or trait mediated (e.g., changes in host phenotype and behavior: Abrams, 1992). Density-mediated effects are mostly a consequence of host resistance and parasite infectivity (Anderson and May, 1978, 1981; May and Anderson, 1978) and are likely to have strong implications for eco-evolutionary dynamics through changes in host population dynamics. Trait-mediated effects, on the other hand, are mostly a function of host phenotypic plasticity and infection-associated pathology and can very quickly alter the per capita strength and direction of a host's interspecific interactions (Wootton, 1994; Werner and Peacor, 2003; Hatcher et al., 2006). Thus, the extent and manner by which parasites influence eco-evolutionary dynamics will depend on their effects on host population properties (density-mediated effects) but also on how and to what extent they influence host phenotype (trait-mediated effects).

The effects of parasites on eco-evolutionary dynamics are typically tested in two basic ways. First, replicated and controlled experimental work in the laboratory is particularly good at revealing causation—but is unrealistic in its removal of extrinsic factors that can shape host-parasite interactions and eco-evolutionary dynamics (Wood et al., 2007). Second, correlative studies in the field provide the realism of showing associations in the context of natural variation in extrinsic factors—but they cannot effectively inform causation (Poulin, 1999; Werner and Peacor, 2003; Hatcher et al., 2006). Thus, *to test for causality in nature*, which is ultimately what we need to understand (Hendry, 2017), we need experimen-

[1] Department of Biology, McGill University, 1205 av. Docteur-Penfield, Montreal, Quebec, Canada; Email: (FPJ) felipe.perezjvostov@mail.mcgill.ca. Send reprint requests to FPJ.

[2] Redpath Museum, McGill University, 859 Sherbrooke Street West, Montreal, Quebec, Canada.

[3] Institute of Parasitology and Centre for Host-Parasite Interactions, McGill University, 21111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, Canada.

tal manipulations of parasitism in nature. In particular, the introduction of a parasite into a previously parasite-free host population would allow quantification of natural density- and trait-mediated effects on the host, as well as potential changes in other members of the community.

Guppy (*Poecilia reticulata* Peters, 1859) hosts and their *Gyrodactylus* sp. (hereafter *Gyrodactylus*) monogenean ecto-parasites are well positioned for such experiments. First, parasite trait-mediated (i.e., growth) and density-mediated (i.e., mortality) effects have been documented in the laboratory and in mesocosms. For example, heavily infected guppies show increased lethargy (van Oosterhout et al., 2003), erratic swimming (Hockley et al., 2014), reduced feeding (Houde and Torio, 1992; López, 1998; van Oosterh-out et al., 2003), and/or reduced reproductive success (Kennedy et al., 1987; López, 1998, 1999; Kolluru et al., 2009; van Oosterhout et al., 2007). In addition, guppies exposed to *Gyrodactylus* show a reduction in growth, suggesting an energetic trade-off in mounting an immune response (Pérez-Jvostov et al., 2012, 2015). These effects often translate into strong changes in guppy population dynamics in laboratory settings (Scott, 1982; Scott and Anderson, 1984; Cable and van Oosterhout, 2007).

Second, another member of the fish community is an obvious candidate for considering cascading non-target effects of host-parasite interactions. The killifish (*Rivulus hartii)* competes with guppies for food, such as aquatic invertebrates, and shelter (Grether et al., 2001; Zandonà et al., 2011). Moreover, larger guppies can prey on juvenile killifish and large killifish can prey on all but the largest guppies (Seghers, 1967; Gilliam et al., 1993; Mattingly and Butler, 1994). Conveniently, the upper reaches of many streams contain only guppies and killifish (Gilliam et al., 1993). In this simple system with strong interactions, parasite-mediated effects on guppies would be expected to influence killifish.

Third, opportunities abound for experimental manipula-tions in nature. For instance, many previous studies have introduced guppies from high-predation environments to previously guppy-free low-predation environments (Reznick and Bryga, 1987; Gordon et al., 2009; Handelsman et al., 2014). The same types of waterfalls that separate these predation communities, and thus facilitate experimental introductions, can also separate parasite communities. In particular, *Gyrodactylus* tends to be common on guppies in downstream sites but can be entirely absent from upstream populations (Martin and Johnsen, 2007; Gotanda et al., 2013). Thus, guppies from parasitized populations can be cleared of their parasites, introduced to previously guppy-free sites, and then allowed to evolve without parasites (Dargent et al., 2013). Conversely, *Gyrodactylus* parasites can be introduced into previously *Gyrodactylus*-free guppy popula-tions to test for eco-evolutionary outcomes—the approach we take in the present study.

Our experiment.—We introduced *Gyrodactylus* into two previously *Gyrodactylus*-free guppy populations, simulta-neously maintaining *Gyrodactylus*-free guppy populations just upstream. Our first prediction was that *Gyrodactylus* would alter population-level parameters for guppies such as survival, biomass, abundance, and/or density. Our second prediction was that *Gyrodactylus* would alter individual-level parameters for guppies, such as their size and life history. Our third prediction was that effects of *Gyrodactylus* on guppies would cascade to influence cohabiting *Rivulus*. Our fourth

prediction was that effects would differ between the two "replicates" (i.e., effects are context dependent) given variation between them in environmental conditions, host genotypes, and parasite genotypes.

MATERIALS AND METHODS

Experimental design.—The experiment was implemented in a replicated BACI (before-after:control-impact) design (Stewart-Oaten et al., 1986). Two initially *Gyrodactylus*-free tributaries were each divided into two adjacent sections: a *Gyrodactylus*-introduction section and an upstream *Gyrodactylus*-free control section. Prior to parasite introduction ("pre-intro-duction phase"), the demography and life history of guppies and *Rivulus* was assessed through mark-recapture methods in both sections. Eight months after the parasite introduction ("post-introduction phase"), an identical second mark-recap-ture experiment was performed to measure the same parameters, again in both sections. With this BACI design, we were able to assess the ecological effects of the addition of a novel parasite in nature.

Experimental protocol.—We studied two genetically distinct populations of guppies (Crispo et al., 2006; Fraser et al., 2009)., each inhabiting a different *Gyrodactylus*-free stream on the north slope of the Northern Mountain Range in Trinidad. One stream was in the Paria River watershed and the other was in the Marianne River watershed. In both streams, a waterfall at the lower end served as a barrier preventing the upstream migration of guppies (and therefore *Gyrodactylus*) from populations further downstream.

We first sought to confirm the presence of *Gyrodactylus* below each waterfall and their absence above. To do so, 100 guppies from each stream were collected. Each fish was anesthetized in a buffered solution of 0.02% tricaine methanesulfonate (MS-222), and its entire surface was scanned through a dissecting microscope, the standard method for quantifying prevalence of *Gyrodactylus* and intensity of infection (Scott, 1982). Samples of 100 fish have proven reliable for determining presence/absence of *Gyro-dactylus* in many studies (Martin and Johnsen, 2007; Gotanda et al., 2013), and results for one time period are highly repeatable in other time periods (Fraser et al., 2009; Gotanda et al., 2013). Immediately below the waterfalls, infection prevalence (percentage of fish that were infected) was 75% in the Marianne (mean intensity = 5.1 + 3.5 SD parasites per fish) and 32% in the Paria (1.4 + 1.0). By contrast, no *Gyrodactylus*-infected fish were found above the waterfall in either river. Above the waterfall, we divided each stream into a downstream "experimental" section of five adjacent pools (where we would later introduce *Gyrodactylus*) and an upstream "control" section of five adjacent pools (where we would not introduce *Gyrodactylus*). In each case, we partially isolated the control section from the experimen-tal section by creating a 50 cm high barrier made of large rocks, gravel, and fallen tree trunks. The goal of this barrier was to prevent (or at least reduce) any potential upstream spread of guppies and therefore *Gyrodactylus*. For each pool at each capture period (details below), we estimated the volume of each pool by multiplying pool area by the average pool depth.

Before parasites were introduced into the experimental sections (see below), we used mark-recapture methodology to calculate growth and of fish from five control and five experimental pools in each river. We used butterfly nets to

collect all fish from each pool, as done in previous studies (Reznick et al., 1996, 2001). We separated all captured fish by pool and species, and held them overnight in the buckets prior to transportation to our Trinidad laboratory the next morning. In the laboratory, the fish were held in pool/ species/sex-specific aquaria and, within two days, were scanned for parasites (as above), weighed (nearest 0.001 g), and individually marked using Visible Implant Elastomer dyes (Northwest Marine Technology Inc.). These procedures are standard for guppies (Gordon et al., 2009; Weese et al., 2010). None of the collected guppies were infected with *Gyrodactylus*, further confirming that these tributaries were *Gyrodactylus*-free. After two days of recovery, all guppies and *Rivulus* were transported back to the field and released into their original pool of capture. One month later, we again collected all the fish, identified them using their individual marks, scanned them for parasites (again none were found), and weighed them as described above. In addition, we quantified life-history traits for guppies (embryonic mass and number of embryos) by dissecting 25 randomly-selected recaptured adult females from each of the introduction and the control sections in each stream.

After the recapture period, we introduced *Gyrodactylus* parasites into the experimental section of each stream. To do so, we collected fish from five pools immediately below the waterfall and scanned them for parasites. After identifying infected fish, we manually transferred three parasites onto each of three randomly-selected marked fish from each of the five pools in the experimental section. After confirming (24 h later) that the infection had successfully established on each fish, they were returned to their home pools in the experimental section. Although with this approach we cannot ensure the use of the same species/strain of *Gyrodactylus* across streams, thus reducing variation in infectivity or virulence, as is known to be the case for some species and strains of *Gyrodactylus* (Pérez-Jvostov et al., 2015), the experimental comparison is valid and needs to be done, as we do faithfully replicate invasion events of the local parasites inhabiting these streams.

Eight months after the introduction of *Gyrodactylus* into the Paria stream, the parasite was still present in the experimental pools and—as intended—absent from the control pools. In the Marianne stream, *Gyrodactylus* was still present in the experimental pools but—as not intended— had also spread into the control pools. (The barrier appears to have been insufficient during higher flows.) We therefore explored further upstream to determine, by inspecting captured fish, how far *Gyrodactylus* had spread. Approximately 250 m upstream of our initial control section, a large fallen tree formed a natural barrier that prevented further upstream movement by guppies and thus also excluded *Gyrodactylus*. This new section became our post-introduction control site for the Marianne. A post-introduction mark-recapture experiment, identical to that described above for the pre-introduction phase, was then conducted in both rivers at the originally planned experimental and control sections in the Paria, and at the new Marianne control section.

All the procedures in our experiments were in accordance with ethical norms and approved by the McGill University Animal Use Committee in the protocol No. 2012-7257. Likewise, we will remove the introduced *Gyrodactylus* upon completion of our investigations on their ecological and evolutionary implications.

Statistical analyses.—Population-level survival estimates were calculated using Wilson Score Intervals for binomial counts and proportions (number of fish recaptured/total number of fish at first capture) in the *binom* package in R version 3.1.2. GUI (R Core Development Team, 2011). Although our single recapture period per experimental phase (pre-introduction and post-introduction) dictated that we cannot independently estimate survival and recapture probabilities, previous work has shown that recapture rates in these habitats are extremely high (Reznick et al., 1996; Weese et al., 2010). The survival estimates were calculated independently for each control and experimental section in each experimental phase and compared using the Holm method for multiple comparisons.

Additional population-level parameters were analyzed with generalized linear mixed effects models (GLMMs) in the *nlme* package in R. The response variables, each considered in a separate analysis, were guppy density (number of guppies/ pool volume) and guppy biomass (weight of all guppies/pool volume). Explanatory variables included the fixed effects of river (Marianne versus Paria), experimental treatment (experimental versus control), phase (pre-introduction versus post-introduction), all two-way interactions, and the three-way interaction. Pool was a random factor nested within treatment. We performed the same set of analyses with the same model structure for *Rivulus*.

Individual-level parameters for guppies were analyzed with separate GLMMs for (a) body mass (grams at first capture), (b) change in body mass over the 30-day period between capture and recapture, (c) female reproductive effort (change in body mass divided by the total mass of embryos at the time of recapture), (d) number of embryos at recapture, and (e) total embryonic mass at recapture. Fixed effects were river of origin (Marianne versus Paria), experimental treatment (experimental versus control), phase (pre-introduction versus post-introduction), and their interactions as above. Sex was included as a fixed effect for analyses of body mass and change in body mass. Pool of origin was included as a random factor nested within treatment. Mean parasite load (individual parasite load averaged between capture and recapture) and body mass at capture were entered as covariates, but mean parasite load was removed owing to non-significance. We fit a similar model for growth of *Rivulus*, with the exception that sex was not included as a main effect because the sex of *Rivulus* was often unknown.

RESULTS

We collected a total 1579 guppies (981 in the Marianne stream and 598 in the Paria stream) and 513 *Rivulus* (285 in the Marianne and 228 in the Paria; Table 1). Almost complete turnover of individuals occurred between the pre-introduction and post-introduction phases in both rivers, with the exception being one female in the Paria stream. *Gyrodactylus* successfully established in both the Paria (post-introduction capture prevalence = 25.2% and mean intensity = 3.1 worms/ infected guppy; post-introduction recapture prevalence = 18.6% and mean intensity = 2.7 worms/infected guppy) and the Marianne River (post-introduction capture prevalence = 27.0% and mean intensity = 2.3 worms/infected guppy; post-introduction recapture prevalence = 16.9% and mean intensity = 0.5 worms/infected guppy). The maximum parasite load on an individual fish was 12 in the Paria and 19 in the Marianne. Such infection levels in the experimental populations were similar to those seen in other wild guppy

Table 1. Descriptive statistics of guppy and *Rivulus hartii* populations before and after the introduction of *Gyrodactylus* ectoparasites. Each previously *Gyrodactylus*-free stream was divided into Control and Experimental sections. Two mark-recapture surveys were performed in all sections: one before the introduction of *Gyrodactylus*, and one eight months after the introduction.

Guppies

River	Treatment	Phase	Total number of fish at first capture	Total number of fish at second capture	Number of recaptures	Percentage recaptured (%)	Number of new collections
Marianne	Control	Pre-introduction	89	75	43	48.31	32
	Experimental	Pre-introduction	130	156	74	56.92	82
	Control	Post-introduction	217	183	108	49.77	75
	Experimental	Post-introduction	157	113	40	25.4	73
Paria	Control	Pre-introduction	89	71	32	35.96	39
	Experimental	Pre-introduction	187	132	63	33.69	69
	Control	Post-introduction	32	39	19	59.38	20
	Experimental	Post-introduction	99	113	50	50.51	63

Rivulus

River	Treatment	Phase	Total fish at first capture	Total fish at second capture	Number of recaptures	Percentage recaptured (%)	Number of new collections
Marianne	Control	Pre-introduction	32	33	10	31.25	23
	Experimental	Pre-introduction	27	34	10	37.04	24
	Control	Post-introduction	37	59	14	37.84	45
	Experimental	Post-introduction	54	57	15	27.78	42
Paria	Control	Pre-introduction	24	25	11	45.83	14
	Experimental	Pre-introduction	43	35	9	20.93	26
	Control	Post-introduction	31	45	9	29.03	36
	Experimental	Post-introduction	26	33	5	19.23	28

populations (Martin and Johnsen, 2007; Gotanda et al., 2013), suggesting that our experimental findings would be relevant to the natural context.

The population-level results indicate that the introduction of *Gyrodactylus* could affect guppy survival while having little effect on either density or biomass. In the Paria stream, survival was generally higher in the post-introduction than pre-introduction phases (not significant for the control section), but the treatment and control sections did not differ from each other in either phase (Fig. 1A). In the Marianne stream, pre-introduction survival was similar in the experimental and control sections and did not change for the control section post-introduction. However, survival was much reduced in the post-introduction experimental section (Fig. 1B). For guppy density and biomass at the population level, some differences also were detected among particular river/treatment/phase combinations (Table 2). In the Paria stream, guppy density and biomass decreased dramatically from the pre-introduction to the post-introduction phases, but this decrease was similar in the control and experimental sections (Fig. 2). In the Marianne stream, guppy density and biomass were similar between the pre- and post-introduction phases, regardless of treatment (Fig. 2).

The individual-level results indicate that the introduction of *Gyrodactylus* dramatically decreased female guppy growth in the Paria but not the Marianne, and that the other measured traits were not influenced in either river. Guppy body mass increased from the pre-introduction to post-introduction phases in the Paria, whereas decreases were seen in the Marianne; yet in no case did the pre-to-post-introduction change differ between control and experimental sections (Table 3, Fig. 3). For the change in mass (i.e., growth), one response to parasitism was evident—Paria females showed increased growth from pre-introduction to

post-introduction phases in the control section but showed decreased growth in the experimental section (Table 3, Fig. 3). This change reflected no difference in growth between control and experimental sections in the pre-introduction phase but a large difference (slower growth in the experimental section) in the post-introduction phase. For the various life history traits, several effects of river were evident (Table 3), but in no case was an effect of parasitism evident.

We could not detect any appreciable effect of any of the investigated explanatory variables on any of the investigated response variables in killifish. As a result, no data are shown for *Rivulus*.

DISCUSSION

Parasite-mediated effects on wild guppies were context (stream) dependent, differing between the two introduction experiments. For instance, Marianne (but not Paria) guppies showed a decrease in survival, whereas Paria (but not Marianne) females showed a decrease in growth. Despite these few stream-specific effects, the broadest interpretation is that parasitism did not strongly influence individual-level (trait-mediated) or population-level (density-mediated) parameters, such as guppy size, guppy biomass, and reproductive allocation. Moreover, coexisting killifish did not appear to be influenced by the introduction of these guppy-specific ectoparasites. These outcomes clearly show that effects observed in laboratory or mesocosm settings are not necessarily evident in nature, although it is also possible that our experiment was not optimally designed to reveal such effects. For instance, we looked only eight months after introducing *Gyrodactylus*, whereas the strongest effects might have been observed on shorter or longer time scales. In addition, natural communities are subject to intermittent environmental conditions that might modify the effects of
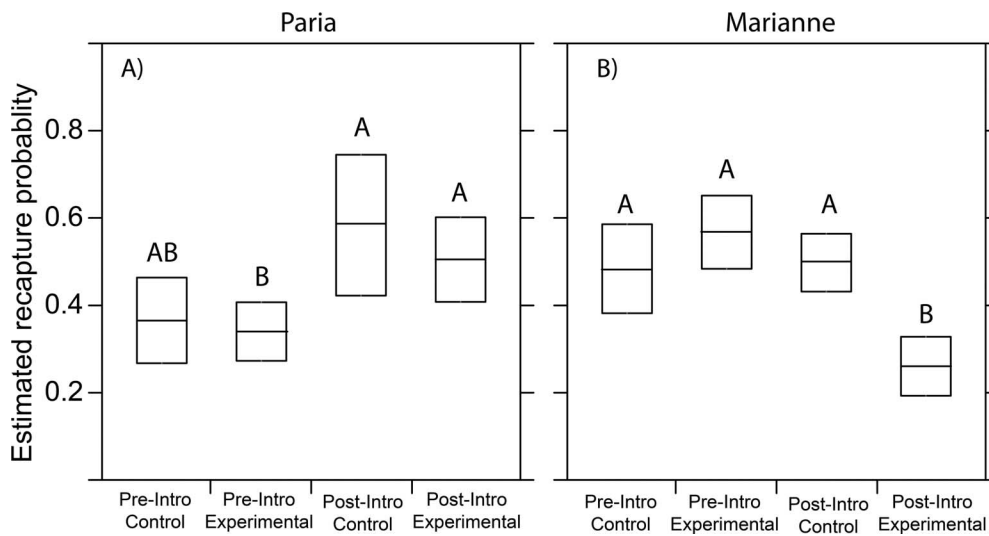
Fig. 1. Normal approximations for 95% binomial confidence intervals on guppy survival before and after the introduction of *Gyrodactylus* ectoparasites. Confidence intervals were calculated using the Wilson Score Interval.

Gyrodactylus. For example, our study was conducted during the wet season, whereas guppy densities are higher in the dry season (Reznick and Endler, 1982; Rodd and Reznick, 1997; Grether et al., 2001), which might enhance *Gyrodactylus* epidemics. Setting aside these potential limitations, we now discuss our results in more detail, especially in relation to how researchers should infer the eco-evolutionary effects of parasitism.

Gyrodactylus *have limited density-mediated effects on gup-pies*.—We can see several possible explanations for why the introduction of *Gyrodactylus* had only modest (Marianne stream) or non-existent (Paria stream) density-mediated effects on otherwise natural guppy populations. First, guppy densities might have been too low (average of 10 guppies/ m^3) to allow the explosive *Gyrodactylus* epidemics that are so detrimental to lab-reared guppies. Indeed, infection prevalence and intensity in our experiment was much lower than that typically seen in the laboratory or in mesocosms (Cable and van Oosterhout, 2007; Pérez-Jvostov et al., 2012). Second, the introduced *Gyrodactylus* could have had low infectivity or virulence, as is known to be the case for some species and strains of *Gyrodactylus* (Cable and van Oosterhout, 2007; Pérez-Jvostov et al., 2015). Third, the guppies could have had high resistance or tolerance to *Gyrodactylus*, as is known to be the case for some guppy populations (Dargent et al., 2013). Fourth, potential effects of *Gyrodactylus* might have been swamped (or overwhelmed) by other mortality agents, such as killifish and *Macrobrachium* prawns (Rodd and Reznick, 1991; McKellar et al., 2009), kingfishers

(Templeton and Shriner, 2004), flooding (Weese et al., 2010), or inter-specific competition (Grether et al., 2001). Fifth, under density limitation, increased mortality owing to *Gyrodactylus* might simply increase survival of the remaining guppies, leading to no net effect on demography. Sixth, increased mortality could be offset by increased reproductive output by mothers that then grow faster and larger (de Roos et al., 2007). Finally, our study populations were "open," and so increased mortality from *Gyrodactylus* could have been compensated by increased immigration from further upstream.

In short, we found that a specialized parasite did not have strong density-mediated effects on naive host populations in nature, a result that seems to contradict *a priori* expectations based on laboratory and mesocosm work in multiple taxa (Minchella and Scott, 1993; Lafferty et al., 2008). We therefore argue that it is not safe to assume that a common ecotoparasite known to have debilitating effects in controlled settings will necessary have population-level consequences in the natural environment. As for the specific reason why effects were minimal, we can postulate many potential reasons (as above), although we cannot discriminate among them in the present study. However, as revealed by this causal ambiguity, it is clear that many potential factors that are quite plausible in nature might complicate or confound simple expectations from theory or laboratory work. Hence, we need more studies in nature if we are to understand when, how, and why (or why not) parasites will influence the dynamics of their hosts.

Table 2. Analyses for density-mediated effects of *Gyrodactylus* on guppies. Density and biomass were calculated for each pool during the mark and recapture collections prior to and after the introduction of *Gyrodactylus* into the experimental section of the stream. Significant *P* values highlighted in bold.

Explanatory variable	Guppy density		Guppy biomass	
	F (df)	P	F (df)	P
River	3.363 (1,41)	0.073	1.249 (1,41)	0.270
Phase	2.459 (1,41)	0.124	1.028 (1,41)	0.316
Treatment	0.040 (1,41)	0.844	0.036 (1,41)	0.852
River X Phase	6.374 (1,41)	**0.015**	3.631 (1,41)	0.063
River X Treatment	9.141 (1,41)	**0.004**	11.579 (1,41)	**0.001**
Phase X Treatment	0.064 (1,41)	0.801	0.082 (1,41)	0.775
River X Phase X Treatment	0.457 (1,41)	0.502	0.076 (1,41)	0.783
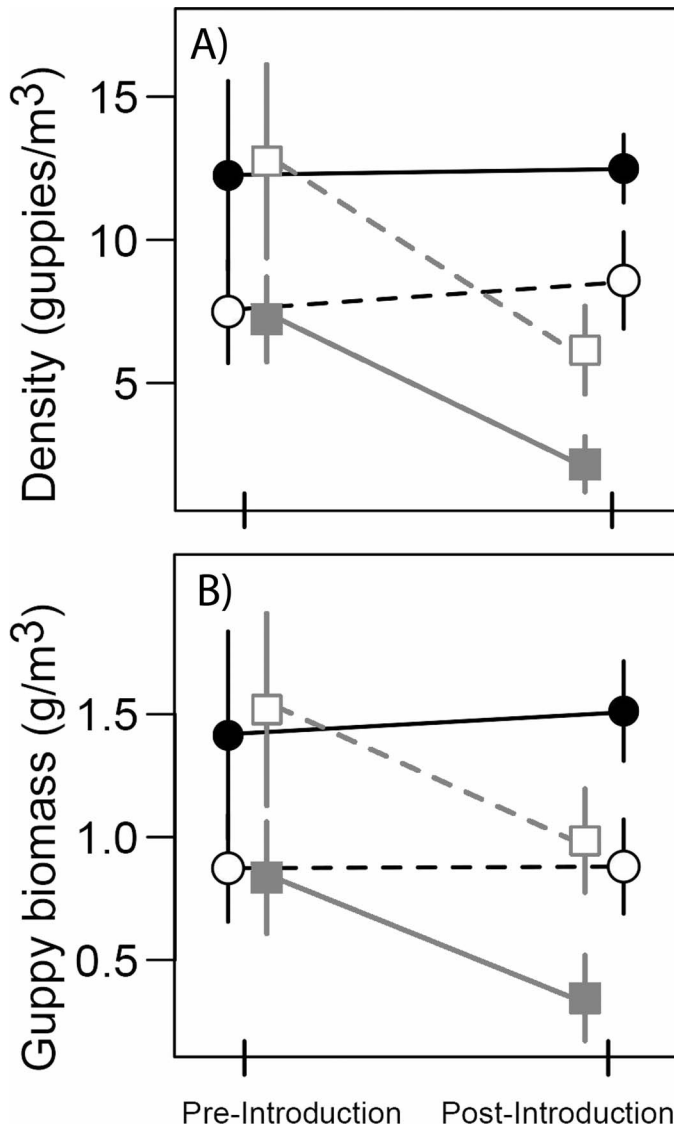
Fig. 2. Least Square Means for guppy density (A) and biomass (B) before and after the introduction of *Gyrodactylus* ectoparasites. Error bars represent standard errors. Symbols represent Paria Control (■), Paria Experimental (□), Marianne Control (•), Marianne Experimental (○).
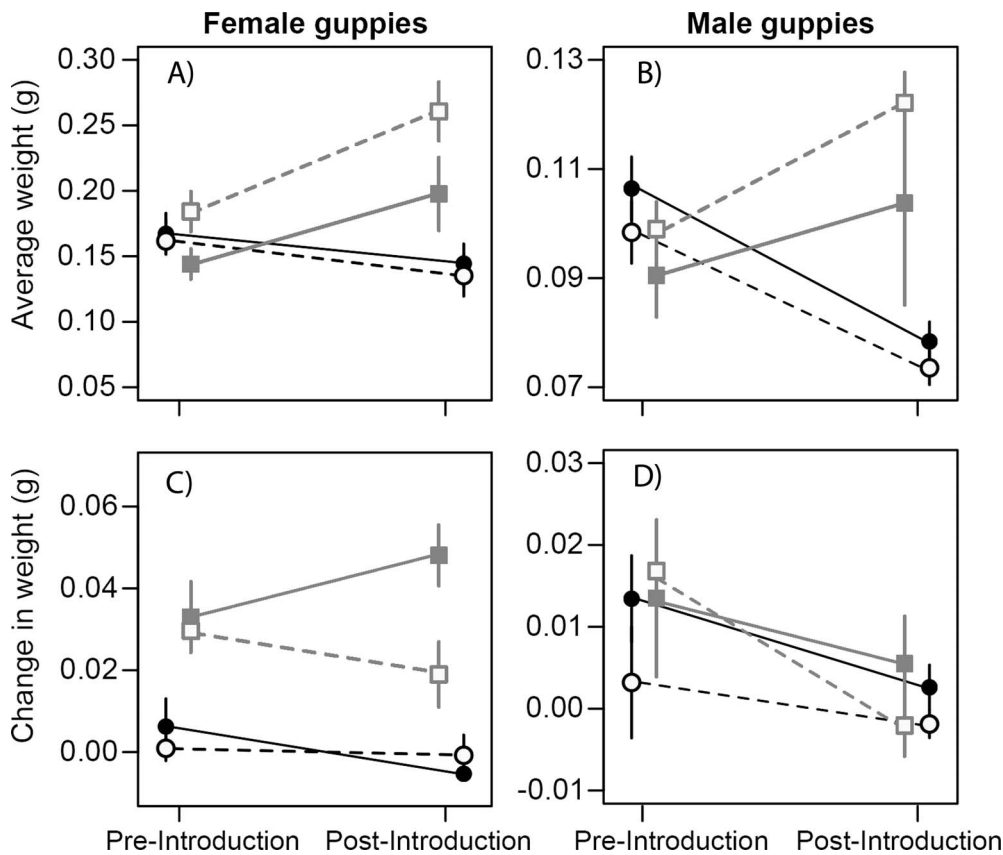
Table 3. Analyses for trait-mediated effects of *Gyrodactylus* on guppies using general linear mixed effects models. Traits were calculated on individual fish during the mark-recapture collections prior to and after the introduction of *Gyrodactylus*.

Explanatory variable	Guppy mass F (df)	P	Change in mass F (df)	P	Total embryonic mass per female F (df)	P	Reproductive effort F (df)	P	Number of embryos F (df)	P
River	9.480 (1, 441)	**0.022**	91.224 (1, 440)	**<0.001**	2.146 (1, 133)	0.145	46.788 (1, 133)	**<0.001**	8.720 (1, 133)	**0.004**
Phase	5.019 (1, 441)	**0.025**	19.828 (1, 440)	**<0.001**	0.174 (1, 133)	0.677	0.134 (1, 133)	0.715	1.035 (1, 133)	0.311
Treatment	0.259 (1, 441)	0.611	5.008 (1, 440)	**0.026**	0.019 (1, 133)	0.89	0.862 (1, 133)	0.355	0.565 (1, 133)	0.453
Sex	64.861 (1, 441)	**<0.001**	4.928 (1, 440)	**0.008**	—	—	—	—	—	—
Guppy weight	74.669 (1, 441)	**<0.001**	74.669 (1, 440)	**<0.001**	47.656 (1, 133)	**<0.001**	5.804 (1, 133)	**0.017**	25.213 (1, 133)	**<0.001**
River X Treatment	6.502 (1, 441)	**0.011**	0.576 (1, 440)	0.448	1.047 (1, 133)	0.308	0.292 (1, 133)	0.59	1.428 (1, 133)	0.234
River X Phase	20.557 (1, 441)	**<0.001**	5.499 (1, 440)	**0.02**	0.175 (1, 133)	0.677	0.059 (1, 133)	0.809	1.180 (1, 133)	0.279
Treatment X Phase	0.259 (1, 441)	0.61	0.242 (1, 440)	0.623	1.225 (1, 133)	0.27	1.459 (1, 133)	0.229	0.076 (1, 133)	0.784
River X Treatment X Phase	0.184 (1, 441)	0.667	6.317 (1, 440)	**0.012**	0.454 (1, 133)	0.502	1.115 (1, 133)	0.293	1.930 (1, 133)	0.167

***Gyrodactylus** have limited trait-mediated effects on guppies.*— The introduction of *Gyrodactylus* reduced female guppy growth rates in the Paria (Fig. 3C, D) despite an overall reduction in guppy density (Fig. 2A)—a result that matches our previous mescocosm experiments conducted with a diversity of populations (Pérez-Jvostov et al., 2012, 2015). In each of these studies, reductions in growth were not due to individual infection status (number of parasites on each fish), but rather the overall presence of *Gyrodactylus* in the system, suggesting a trade-off between growth and a prophylactic immune response (Sadd and Schmid-Hempel, 2009).

Despite this one specific result that matched previous work in controlled settings, the more general conclusion should be that parasite introduction did not have strong effects on size, growth, or reproduction of naïve guppies in nature. This discrepancy between experimental "venues" (*sensu* Skelly and Kiesecker, 2001) was particular striking for Marianne guppies, which showed parasite-induced growth reductions in mesocosms (Pérez-Jvostov et al., 2012) but not in nature

Fig. 3. LS Means for guppy mass and growth before and after the introduction of *Gyrodactylus* ectoparasites. Error bars represent standard errors. Symbols represent Paria Control (■), Paria Experimental (□), Marianne Control (•), Marianne Experimental (○).

(Fig. 3). Most potential *post hoc* explanations for this discrepancy between venues match those outlined in the previous section on (the general lack of) density-mediated effects. In addition, individual guppy hosts for which the effects of parasites were most detrimental might have perished more quickly, leaving us to sample only the hosts for which the effects of parasites were least detrimental. This last interpretation matches one explanation for why field surveys of un-manipulated populations do not reveal associations between guppy color and parasite loads (Martin and Johnsen, 2007; Gotanda et al., 2013).

Gyrodactylus *infecting guppies have no effects on coexisting killifish.*—If parasites influence the traits or population dynamics of hosts, they would be expected to have cascading effects on other species with which the host interacts (Holt, 1977). However, we did not record any influence of the introduction of *Gyrodactylus* on killifish, a species with which guppies have a number of detrimental interactions, both as competitors and predators/prey (Gilliam et al., 1993; Fraser and Lamphere, 2013). Yet, in light of the above-described minimal effects on guppies, this non-effect on killifish is not so surprising: if the host is not much affected, perhaps other community members will not be either.

As an interesting alternative, killifish might actually reduce the effects of *Gyrodactylus* on guppies. Indeed, we previously used mesocosm experiments to show that guppies exposed to both *Gyrodactylus* and killifish show intermediate growth between guppies exposed to *Gyrodactylus* but not killifish (decreased growth) and guppies exposed to killifish but not *Gyrodactylus* (increased growth; Pérez-Jvostov et al., 2016). The likely explanation is intra-guild predation arising when large killifish prey on small guppies. Specifically, intermediate guppy growth in the joint presence of killifish and

Gyrodactylus could represent a suitable compromise between growing to a predator-resistant body size while also reserving resources for a parasite-induced immune response. These possibilities further highlight the need for field experiments that assess the effects of parasites in the context of all other interacting factors typical in nature.

Context dependence—*differences between the two introductions.*—As repeatedly noted, different results were obtained in the two different parasite introductions. We do not know the specific reason for this context dependence, but several possibilities seem likely. First, different histories of selection, genetic bottlenecks, drift, and founder events may all influence how guppy populations respond to the introduction of the novel *Gyrodactylus* parasite (Willing et al., 2010). Second, different populations of *Gyrodactylus* have undergone different co-evolutionary trajectories with their sympatric guppy hosts in different rivers, resulting in strong population-level variation in infectivity and virulence (Pérez-Jvostov et al., 2012, 2015). Third, different environmental characteristics in the two rivers could have modified host-parasite interactions. For example, low-predation tributaries such as those in our experiment have been shown to differ in resource levels, predator densities, flow rates, and primary productivity (Grether et al., 2001; Millar et al., 2006; McKellar et al., 2009). Any of these factors could—in principal—influence the cost of being parasitized or of mounting and deploying defenses against parasites.

In short, the context in which ecological interactions play out is a major factor driving eco-evolutionary dynamics and seems to be a reoccurring theme in the Trinidadian guppy system (Fitzpatrick et al., this volume, 2017; Gordon et al., this volume, 2017). The only way in which we can disentangle and understand this context dependence is

through additional replicated and controlled experiments *in nature*.

Conclusions.—In our experiment, the effects of parasites in nature were context specific and generally weak. These outcomes highlight the strong difference between the effects of parasites seen in natural host populations (relatively weak and inconsistent effects) versus laboratory experiments where conditions are controlled (relative strong and consistent effects). This discrepancy mirrors the findings of previous meta-analyses that showed how "venue" (lab versus mesocosm versus natural) has important effects on the outcome of ecological experiments (Skelly and Kiesecker, 2001). Moreover, although context dependence has certainly been demonstrated in laboratory and mesocosms experiments, our results suggest that such variation could be especially strong in nature. Another important message from our study—following from the above—is that the effects of a common and presumed deleterious parasite can be rather minimal in nature, even when the hosts are naïve with respect to the parasites. This surprising outcome demands further studies of why seemingly important parasites are not always so. In summary, our study highlights the complexity behind host-parasite interactions in the wild, the importance of local processes (context dependence) in influencing such dynamics, and the pressing need for more experiments in nature.

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