Experimental evaluation of predation as a facilitator of invasion success in a stream fish

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Abstract. Predator–prey relationships in poikilotherms are often size dependent, such as when adults of two interacting species are capable of eating juveniles of the other species. Such bi-directional predation can be important during the establishment and spread of an invading species, but its role remains poorly understood. Using a combination of laboratory and mesocosm experiments and field introductions, we demonstrate that guppies, *Poecilia reticulata*, prey on juvenile killifish, *Rivulus hartii*, and thereby facilitate their establishment in the habitat of a potential predator. Laboratory studies found that mature guppies can consume larval *Rivulus*, and experimental stream studies showed that guppies reduced the number of *Rivulus* surviving from eggs. Growth trials found that interspecific competition, while significant, cannot account for the declines in the survival of juvenile *Rivulus* seen in field surveys. Finally, a field experiment, in which guppies were introduced into previously guppy-free stream reaches, resulted in a marked reduction in the abundance of juvenile *Rivulus* relative to guppy-free controls. Together, these results indicate that reducing the native *Rivulus* population represents an important mechanism promoting guppy invasion success.

Key words: ecological niche construction; field experiment; intraguild predation; invasion success; mesocosm experiment; Poecilia reticulata; *realized niche;* Rivulus hartii; *size-specific predation; size-structured populations; Trinidad.*

Introduction

The direction of predation is often life-stage dependent in taxa with indeterminate growth (Persson 1988, Polis 1988, Janssen et al. 2002). Stage-dependent reversals between predators and their prey (Polis 1988) add complexity to the food web and can have significant demographic (Walters and Kitchell 2001, Fauchald 2010) and evolutionary (e.g., Walsh et al. 2011) consequences. Such mutual predation could be especially important in determining the success and rate of spread of invading species, because it could not only provide the invader with a source of energy but also, over time, reduce its mortality due to predation. Similarly, both empirical (Crowder and Snyder 2010) and theoretical (Hall 2011) studies indicate that consuming a resident competitor can facilitate the establishment and spread of an invader. As a result, if the invader's predation on juvenile residents were strong enough to reduce the density of the resident, the invader's realized niche might be initially restricted but expand over time. In such a case, the invader could eventually exclude the resident, or the two species could coexist with the resident at densities reduced from pre-introduction levels. Here, we use empirical studies to examine the complex interaction between an invading fish species and a resident that acts as both a potential predator and competitor.

Mutual predation may explain a conundrum involving the interaction of the guppy, *Poecilia reticulata*, and its intraguild predator, the killifish, *Rivulus hartii*. Field and laboratory studies show that *Rivulus* (maximum total length ~100 mm) can prey upon immature guppies (Seghers 1974, Mattingly and Butler 1994), yet guppies (maximum total length ~45 mm) consistently invade habitats occupied by *Rivulus* when given the opportunity (Magurran 2005, Deacon et al. 2011). In fact, a stream survey found that the presence of guppies reduced the biomass of *Rivulus* in pools by two-thirds relative to adjacent *Rivulus*-only pools, and noted that the difference could not be explained by habitat (Gilliam et al. 1993). The same study used mesocosms to show that guppy presence reduced *Rivulus* growth, consistent with the hypothesis that the decline in *Rivulus* biomass was due to resource competition. From these observations, the coexistence of these species has been viewed as a balance between a stronger predator (*Rivulus*) and a stronger competitor (guppies), as predicted by simple models of intraguild predation (Holt and Polis 1997). However, recent work indicates that guppies affect *Rivulus* life histories in a manner that is inconsistent with resource limitation being the mechanism of guppy impact (Walsh et al. 2011, Furness et al. 2012). Rather, guppies affect *Rivulus* life histories much the same way that piscivorous fishes affect guppy life histories

(Reznick et al. 1990), leading Walsh et al. (2011) to infer that predation by guppies on juvenile *Rivulus* was a significant factor in *Rivulus* evolution.

We estimated the effect of guppies on *Rivulus* larvae survival and overall population structure in order to ask whether mutual predation contributes to the lower biomass of *Rivulus* populations that are sympatric with guppies. We also assessed the impact of each species' density on the somatic growth of the other to infer the relative impact of interspecific competition. To simulate taxa at the initial phase of an invasion, rather than later when co-evolution has occurred, we used naïve *Rivulus* (from stream sections containing *Rivulus* but not guppies or other fish) and guppies from downstream reaches that contained piscivorous fishes, including *Crenicichla alta* and *Hoplias malabaricus* (Magurran 2005). We used a combination of laboratory and mesocosm experiments, plus a field introduction experiment, to test the hypotheses that (1) guppies reduce juvenile survival in *Rivulus*, (2) the effect is strong enough to affect *Rivulus* population structure, and (3) guppy predation shapes habitat use by larval *Rivulus*. Together, these data will assess the role of guppy predation on juvenile life stages of *Rivulus*, which is both a predator and competitor, in facilitating a guppy invasion of *Rivulus*-only stream reaches.

MATERIAL AND METHODS

Laboratory studies

Walsh and Reznick (2009) used life history and demographic data to infer that guppy predation was an important selective factor for *Rivulus*. In order to determine the plausibility of guppy predation on *Rivulus*, we conducted laboratory tests in which adult guppies were presented with juvenile *Rivulus* of a range of sizes. Domestic guppies were maintained on commercial flake food for several weeks prior to use. Guppies were measured for standard length (SL). We hatched larval *Rivulus* from eggs spawned in the laboratory. A test consisted of dropping a larval *Rivulus* 5 cm in front of the guppy and timing capture and ingestion times (i.e., prey no longer visible in guppy's mouth). The test was stopped following ingestion or at 20 minutes if no capture occurred. We analyzed the relationship between ingestion time and the *Rivulus*–guppy size ratio, controlling for sex, by a generalized linear model (GLZ). Both ingestion time and the covariate, size ratio, were $\log_{10}$-tranformed to normalize the dependent variable and to linearize their relationship. Sex was a categorical predictor. This analysis and all subsequent factorial analyses were done with Statistica version 9.1 (Statsoft 2009).

Experimental stream studies

We used experimental streams to quantify the effect of guppies on the survival of early life stages of *Rivulus* in more complex habitats. The experimental stream facility was located at Ramdeen Stream in Trinidad's Northern Range Mountains (fully described in Fraser et al. [1995]). The facility consisted of multiple flow-through channels that received water from a nearby, first-order stream. A thin layer of gravel and fine particulate organic matter formed the substrate, and a natural algal flora and invertebrate fauna developed on the walls and bottoms of the channels. Flow rates were adjusted to maintain water depths at 5–10 cm. The uncovered channels were open to the input of both invertebrates and organic matter from the overhead canopy.

We conducted two sequential trials of this experiment; the first set had six paired replicates and ran for 89 days, the second had five paired replicates and ran for 66 days. Each channel was 0.3 m deep × 0.4 m wide × 2.6 m long. We divided each stream channel into two 1.3-m-long sections, resulting in one upstream and one downstream section. We initiated trials with eggs (*Rivulus*-only, Ramdeen Stream origin) spawned in the laboratory. Near-term eggs were stocked in the sections by placing them in petri dishes containing gravel, pressing the covered petri dish into the channel substrate, and removing the cover. We stocked 26 *Rivulus* eggs per section in the first trial and 15 eggs per section in the second. Three (Trial 1) or four (Trial 2) adult female guppies were stocked into the paired sections, alternating the order of the paired treatments in the stream lines. The total length (TL) of the female guppies was similar between trials (Trial 1, 34.6 ± 3.74 mm [mean ± SD]; Trial 2, 34.9 ± 2.62 mm). At the end of each trial, each section was searched for fish.

A significant reduction in survival would be consistent with direct predation by guppies on *Rivulus*. The experiments ran for less time than the minimum required for a second generation of *Rivulus* to be present at the end of either trial (~106 days; Walsh et al. 2011), so all *Rivulus* present at the end of the trial were considered to have hatched from the stocked eggs. To test the prediction that guppies would reduce survival of larvae against the null, we used a one-tailed, paired-samples *t* test. The paired-sample approach tests the difference within each pair of proportions, rather than the original proportions for the two treatments. As a result, any differences between trials or channels should not bias the test result.

Competition assays

To assess the role of competition in the *Rivulus*–guppy interaction, we conducted growth trials with similar-sized *Rivulus* and guppies. We anchored flow-through, plastic, horticulture pots, 53 cm deep and 40 cm at the base, in Ramdeen Stream. Stream gravel and cobble were placed in the bins one week prior to stocking fish. The bins were open to terrestrial input, and mesh windows below the water line allowed their substrates to re-establish as small invertebrates were free to enter. To simulate the initial stage of an invasion by high-predation guppies, we used naïve *Rivulus* from the *Rivulus*-only section of Ramdeen Stream and guppies

from a downstream high-predation section. We placed up to eight fish in each bin, following a response-surface design (Inouye 2001). Over three trials, we started 54 bins with varying initial numbers of *Rivulus* or guppies, as shown in Appendix Table A1. Mortalities were minimal, but two bins were excluded from the analysis due to complete loss of fish. Trials were started on 16 May 2008, 25 Febrruary 2009, and 8 March 2009, and they ran for 13, 11, and 34 days, respectively. We measured growth by individually marking each fish by injecting a colored elastomer (Northwest Marine Technologies, Shaw Island, Washington, USA) and weighing them at the start and end of each trial. Initial total lengths of *Rivulus* and guppies were 24.0 ± 3.31 mm and 22.4 ± 2.66 mm (mean ± SD), respectively. Young female guppies were used to ensure the resources acquired were applied to somatic growth and not courtship or offspring.

Individual growth constitutes a useful indicator of competition effects in many taxa with indeterminate growth (Bystrom and Garcia-Berthou 1999, Goldberg et al. 1999). For this experiment we analyzed the impact of intra- and interspecific competition on growth (body mass) of each species by fitting a linear model in JMP 9.0.2 (SAS Institute 2011) with trial as a random effect and initial *Rivulus* and guppy mass as fixed effects:

$$\text{Growth}_i = b0 + b1 \times \text{Init}M_i + b2 \times \text{Init}M_j \quad (1)$$

where Growth_i is the per-gram growth in mass (Δ mass $\times$ initial mass^{-1} $\times$ time^{-1}) of species i in a bin and $\text{Init}M_i$ and $\text{Init}M_j$ are the initial mass of conspecifics (i) and heterospecifics (j) in a bin, respectively. Growth_i is analogous to per capita population growth ($\Delta N / N \Delta t$) in Lotka-Volterra competition models, meaning that $b0$, $b1$, and $b2$ can be used to calculate individual growth analogs to Lotka-Volterra parameters α and K:

$$\text{Growth}_i = r_i - \frac{r_i}{K_i} \times \text{Init}M_i - \frac{r_i \times \alpha_{ij}}{K_i} \times \text{Init}M_j \quad (2)$$

where r_i, is the growth rate of species i at low density, α_{ij} is a competition coefficient for the impact on the growth of species i by species j (with the impact of conspecifics, α_{ii}, scaled to 1.0), and K_i is the mass at which species i would experience zero growth in a bin in the absence of species j. We also used these parameter estimates to predict the equilibrium mass ($\hat{M}_i$) for each species (i.e., the combination of masses at which the model predicts zero individual growth for both species):

$$\hat{M}_i = \frac{K_i - \alpha_{ij} \times K_j}{1 - \alpha_{ij} \times \alpha_{ji}}. \quad (3)$$

Effects of substrate and Rivulus origin

We next asked how complex substrates and previous experience with predators might mediate guppy predation effects on *Rivulus*. We used a factorial 2 × 2 × 3 design, with structure (present vs. absent), origin of the *Rivulus* (*Rivulus*-only stream section vs. high-predation stream section), and guppies ($N = 0$, 1, and 2) as predictor variables and *Rivulus* larvae survival as the response. We collected *Rivulus* for experiments from three locations in the Guanapo drainage (see Gilliam et al. [1993] for map), one high predation and two that lacked fish predators. The predator-free sites were in the *Rivulus*-only headwaters, and the high predation *Rivulus* and guppies were collected from a high predation site ~10 km downstream.

The experimental trials were run at Simla research station in Trinidad, under an evergreen forest canopy. The containers were horticulture bins (described in *Competition assays*, above), each containing 19 L of spring water, ~0.3 m deep. For the structure treatments we added a thin layer of gravel, a single cocoa leaf (*Theobroma cacao*), and a single cored, concrete, building brick. The containers in the no-structure treatments received no substrate.

We did four replicates of the factorial design. The first two ran for 22 days, and the second two for 36 days. We spawned *Rivulus* in the laboratory, and stocked the fertilized eggs into the experimental units when they were near term. We placed six (first two replicates) or five (second two replicates) eggs into mops of frayed rope, which mimicked natural nesting material. Containers in the guppy treatments received either one or two female guppies from the high-predation site (trial 1, TL = 31.2 ± 0.28 mm; trial 2, TL = 31.8 ± 0.41 mm [mean ± SD]). We used a generalized linear model to evaluate the factorial design. The relationships between the dependent variable (proportion of surviving *Rivulus*) and predictor variables (number of adult guppies, substrate, and origin) indicated that a normal linear model with an identity link were appropriate for the analysis.

Field introduction

We performed an introduction experiment in which guppies from downstream, high-predation sites were stocked into two, third-order tributaries of the Guanapo River (Caigual and Taylor Streams) that had previously held only *Rivulus*. We used a BACI (Before–After-Control–Impact) design to ask whether stocking guppies in the Introduction reaches would significantly increase the mortality of young *Rivulus* in the field. We inferred a guppy impact on *Rivulus* populations from changes in the size distribution and population estimate in Introduction reaches relative to adjacent guppy-free Control reaches. If guppies prey on *Rivulus* larvae and post-larvae, we would expect that, in the short term, the frequency of small *Rivulus* would decline in the presence of guppies, and thereby shift the size distribution toward a larger mean total length. Alternatively, if guppies primarily affect the growth of juvenile *Rivulus* and not their survival, we would expect a short-term accumulation of *Rivulus* in those smaller size classes (Werner and Gilliam 1984). Over time, compensatory reproduction by, or mortality

of, *Rivulus* might mitigate or even eliminate these shifts (de Roos et al. 2007), but here we examine only the initial response to the guppy introduction.

The Introduction reaches in the Caigual and Taylor streams were each stocked with ~100 guppies with an even sex ratio in April 2009. Control reaches were 50–100 m upstream of the Introduction reaches, above barrier waterfalls that prevented further upstream movement of the introduced guppies. The Introduction reaches in the Caigual and Taylor streams had thalweg lengths of 68 and 48 m, respectively, and the Control reaches of the two streams measured 33 and 25 m, respectively.

Sampling was done after dark when *Rivulus* typically come out from hiding and are easily spotted with headlamps. Searches targeted *Rivulus* over 15 mm TL, which were caught by dipnetting and with minnow traps in deep pools. Each fish captured was measured and given an individual mark using the elastomer (see *Competition assays*, above). We sampled each stream reach for an hour, with each sampler responsible for searching ~25 m of stream length. Searches included both pool and riffle habitats. Our adherence to standardized sampling procedures and sampling times minimized variation in effort across samples, so that the resulting size distributions could be reliably compared (Control vs. Introduction reaches and pre- vs. post-guppy-introduction periods), without assuming that they represented the "true" size distributions. We tested for sampling bias in the size distribution by comparing the capture probability of small ($\leq$35 mm TL) and large (>35 mm TL) *Rivulus*. We used a multi-strata model in Program MARK (version 6.0; White and Burnham 1999). Models that estimated separate capture probabilities for each size class by time period and/or stream reach combination received little support (AIC weight < 0.005), indicating that the observed changes in size distributions were not caused by differences in size-specific capture probabilities.

We sampled streams three times in the months before the introduction (April 2009) and seven times in the first year afterwards. In a BACI design, testing for a time × treatment interaction offers a test for treatment effects while accounting for possible environmental changes over time. Thus, we tested for the effect of the guppy introduction on the size distribution of *Rivulus* using a 2 × 2 ANOVA with time (before vs. after introduction), and treatment (Introduction vs. Control reaches) as predictor variables. The response variable, total lengths of individual *Rivulus*, was $\log_{10}$-transformed for the analysis. Owing to the lack of independence of the size distributions between adjacent sampling periods, we used the size distribution of captured *Rivulus* at the beginning of the introduction (day 0) and one year later (day 352), because we assumed that, over this period, the distributions would have become reasonably independent in a system open to migration, recruitment and mortality.

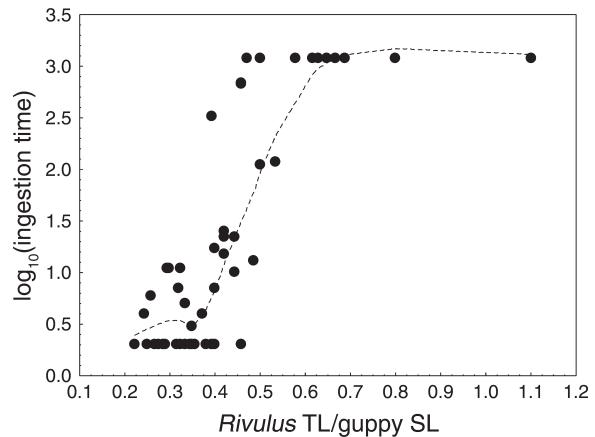We used the mark–recapture data to estimate population sizes in each reach using the POPAN

FIG. 1. Aquarium tests of ingestion times for guppies on larval *Rivulus* as a function of the prey-to-predator (*Rivulus*/guppy) size ratio. SL is the maximum standard length, and TL is total length. The dashed line (lowess smoothing) shows that guppies consumed larval *Rivulus* up to one-half their size. Points above 3.0 on log-scaled *y*-axis indicate failure to consume prey within the allotted test time of 1200 seconds.

parameterization of the Jolly-Seber model in program MARK (White and Burnham 1999). Models estimated the number of *Rivulus* $\geq$30 mm TL, the approximate size at which *Rivulus* become sexually mature. To account for the differences in area among the reaches, we then divided each population estimate for each reach by its base flow, wetted area to yield a density in number per square meter of stream. Finally, to distinguish population changes due to seasonal effects from those due to guppies, we calculated the difference in density (Introduction − Control) over time.

We note that gut contents can provide more direct evidence of predation rates in the field. However, we did not collect gut data from guppies in the field introduction due to concern that taking guppies would jeopardize their establishment.

RESULTS

Laboratory study

All evaluated size classes of guppies (>15 mm long) were able to eat larval *Rivulus* (TL = 7.30 ± 0.39 mm [mean ± SD]). Ingestion times increased sharply, however, as the ratio of *Rivulus* to guppy size increased (Fig. 1) The *Rivulus*/guppy size effect was highly significant (Wald statistic = 30.99, $P < 0.001$), while guppy sex had no effect (Wald statistic = 2.75, $P = 0.251$). Guppies less than 15 mm long could not consume any size class of *Rivulus*, but adult female guppies (SL = 35 mm) successfully ate *Rivulus* as large as 12 mm TL.

Experimental stream study

Rivulus survival (proportion of individuals) was 1.6 times greater in conspecific trials compared to those with guppies present (without guppies, 0.440 ± 0.06 [mean ±

TABLE 1. Results of competition assays between similarly sized *Rivulus* and guppies, using per-gram change in mass (Δ mass $\times$ [initial mass]$^{-1}$ $\times$ time^{-1} with units of g·g^{-1}·[30 days]$^{-1}$) as the response variable.

Parameter†	*Rivulus*†		Guppy‡	
	Mean	SE	Mean	SE
$b0$	**1.798**	0.238	0.290	0.147
$b1$	**−2.155**	0.489	−0.362	0.225
$b2$	−1.212	0.592	**−0.463**	0.186
a ($b2/b1$)	0.562		1.277	
K ($b0/b1$) (g/bin)	0.834		0.799	
$\hat{M}$ (g/bin)	0.834		0.000	

Notes: Parameters are α, the competitive coefficient for impact on growth of species i by species j; K, the mass at which species i experiences zero growth in a bin in the absence of species j; and $\hat{M}$, the equilibrium mass for each species. Standard errors are unavailable for derived parameters α, K, and $\hat{M}$. Boldface values are significant at $P < 0.05$. See *Methods: Competition assays* for details of modeling approach.

† For *Rivulus*, $n = 39$ bins; model $r^2 = 0.493$, and model $P < 0.0001$.

‡ For guppy, $n = 41$ bins; model $r^2 = 0.477$, and model $P < 0.0001$.

SE]; with guppies, 0.273 ± 0.05). In both experimental stream trials, the mean proportions of *Rivulus* surviving from stocked fertilized eggs in the guppy treatment were significantly lower than in the no-guppy treatment ($t = 1.945$, df $= 10$, $P = 0.040$).

Mesocosm studies

In the competition assays, each species exhibited reduced growth in bins with higher densities of both conspecifics and heterospecifics. In fact, the highest-density bins (i.e., eight fish total) apparently exceeded their K (mass at which species i experiences zero growth in a bin in the absence of species j), as they exhibited a net loss of mass during the trial. Both species exhibited positive growth at lower densities, with *Rivulus* nearly doubling in mass (0.930 g/g per 30 days) and guppies adding about 10% per 30 days (0.100 g/g per 30 days). Applying Eq. 1 to the data yielded significant models for both species (*Rivulus*, $r^2 = 0.49$, $P < 0.0001$; guppies, $r^2 = 0.48$, $P < 0.0001$). The competition coefficients (α) indicated that, on average, both species' growth was more affected by a given mass of *Rivulus* than the same mass of guppies (Table 1). As a result, Eq. 3 predicts that, at equilibrium ($\hat{M}$), resident *Rivulus* would exclude invading guppies (Table 1).

Adult guppies significantly reduced survival of larval *Rivulus* in the mesocosm experiments across all treatments (Wald statistic $= 24.50$, df $= 2$, $P < 0.001$; Fig. 2). The analysis also found that substrate presence tended to increase *Rivulus* survival among *Rivulus* of high-predation origin (marginally insignificant origin $\times$ substrate interaction; Wald statistic $= 3.691$, df $= 1$, $P = 0.055$).

Field introduction

Size histograms of the captured fish for both streams show an increase in the relative abundance of small *Rivulus* in the Control reaches in the year following the guppy introduction (Taylor Stream, Fig. 3a vs. 3b and Caigual Stream, Fig. 3e vs. 3f), indicating that favorable
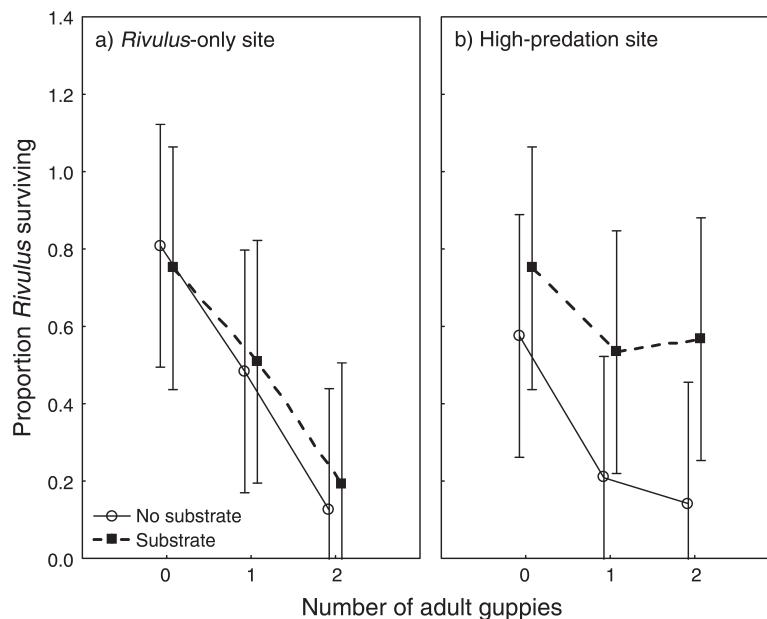

FIG. 2. Mean proportion of *Rivulus* from (a) *Rivulus*-only and (b) high-predation sites that survived in field container experiment relative to the presence of adult guppies and level of substrate structure in the container. Bars represent 95% confidence intervals.
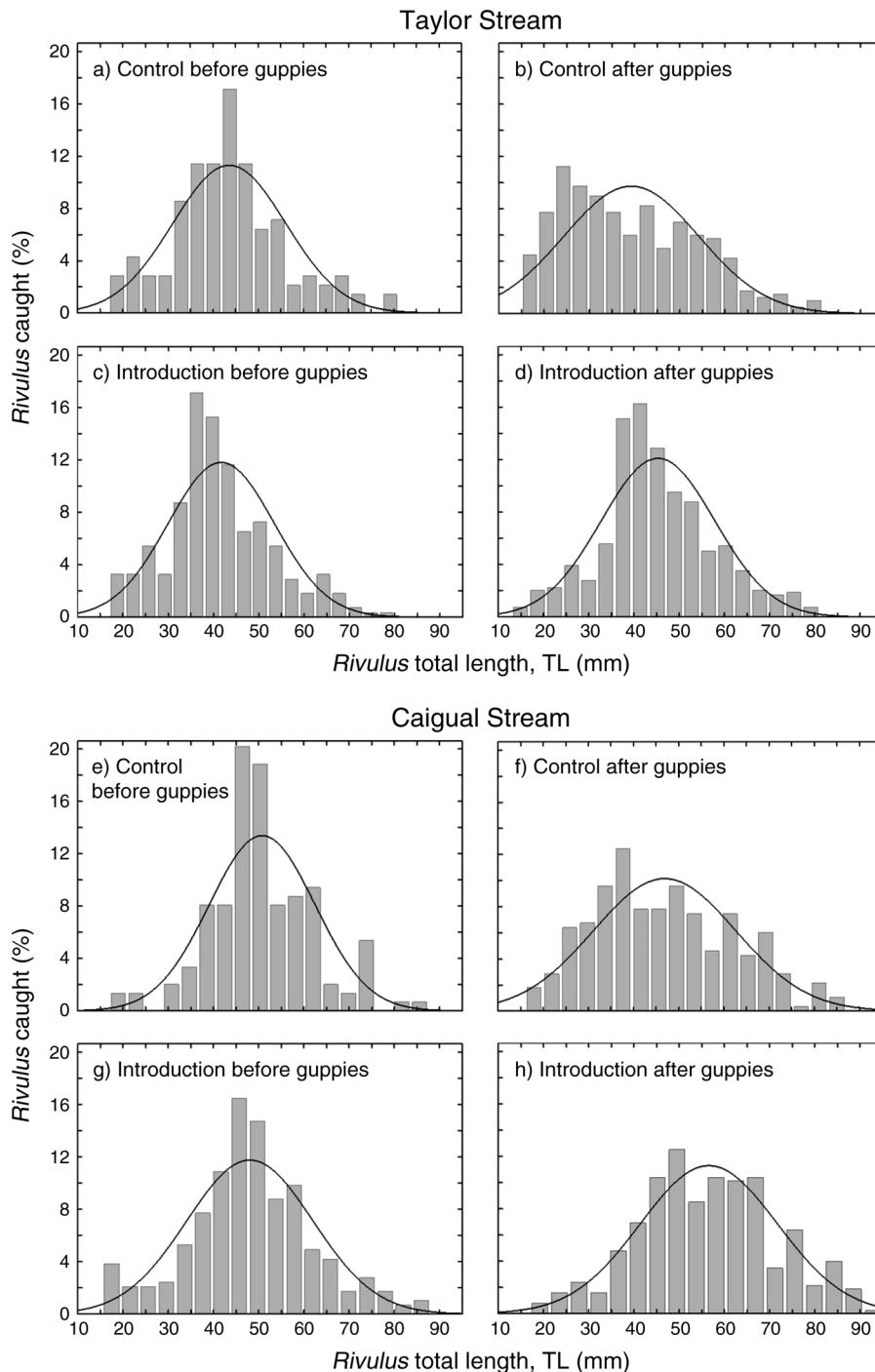
FIG. 3. Size distributions, plotted as percentages of the total *Rivulus* caught in the Control and Introduction reaches of the Taylor and Caigual streams, before guppies were introduced and one year after the introduction. Each distribution is the composite of three sampling periods, each one month apart, taken at the same time of year (January–March) before and after the introduction. A normal fit line is shown for reference.
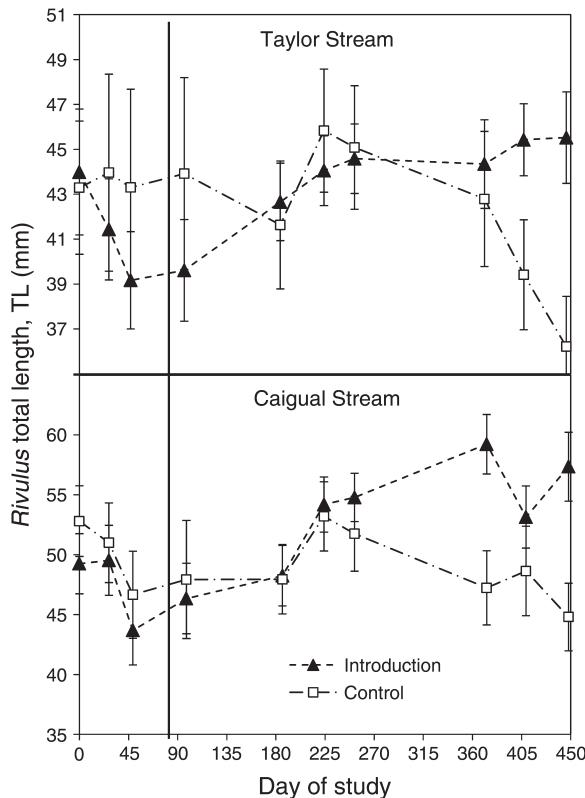
FIG. 4. Field introduction experiment. Mean total lengths (with 95% confidence intervals) of *Rivulus* relative to sampling dates during the 450-day period January 2009 to April 2010. The vertical bar indicates the day of guppy introduction. Day 0 (*x*-axis) is 16 January (Caigual Stream) or 17 January (Taylor Stream).

vs. 3h). The size distribution of *Rivulus* in each of the Introduction reaches shifted toward larger mean size, consistent with the hypothesis that guppy predation reduces the abundance of juvenile *Rivulus*. Prior to the stocking of guppies in the Introduction reaches, neither Introduction–Control pair differed in mean size of individuals (Fig. 4). After the introduction, the means of the size distributions of the Introduction and Control reaches had diverged, due in part to the loss of small *Rivulus* (Fig. 4). The statistical interaction between time period (pre- vs. post-introduction) and reach (Introduction vs. Control) confirms the visible trend in Fig. 4, and is highly significant in both streams (ANOVA interaction effect: Taylor Stream, $F_{1,1081} = 36.9$, $P < 0.001$; Caigual Stream: $F_{1,888} = 34.74$, $P < 0.001$).

Population estimates for adults in each reach before and after the introduction confirm the patterns seen in the size distributions. Control-reach densities increased in both streams, while Introduction reaches experienced no significant increases. As a result, the difference between reaches (Introduction − Control) shifted in the negative direction within a year following guppy introduction (Fig. 5). While *Rivulus* density in the Caigual Introduction reach declined gradually through the year, relative to its Control reach, the Taylor Introduction reach rose early in the year before declining sharply from December 2009 to April 2010.

DISCUSSION

The results of this study support the hypothesis that guppies, and not an environmental correlate of their presence, cause a significant increase in the mortality of juvenile *Rivulus*, especially during the early stages of an invasion. Although interspecific competition or indirect processes could contribute to the negative impact of guppies on *Rivulus*, the evidence presented here and in previous studies of *Rivulus* life history (Walsh et al. 2011) identifies predation as the principal mechanism. We further infer that guppy predation on early life

environmental conditions supported a strong pulse of reproduction. In contrast, the Introduction reaches show either no increase or a decline in the relative abundance of younger age classes a year after the guppy introduction (Taylor, Fig. 3c vs. 3d and Caigual, Fig. 3g
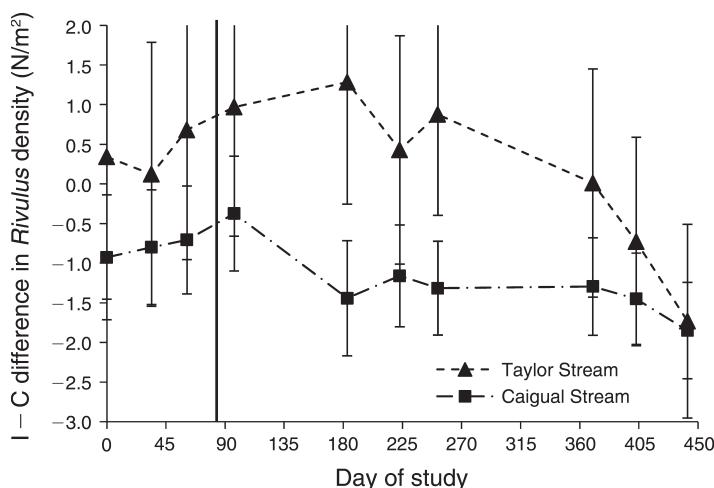

FIG. 5. Population estimates for adult *Rivulus* in the 450-day field introduction experiment from January 2009 to April 2010. *Rivulus* densities are plotted as the difference between Introduction (I) and Control (C) reaches (mean with 95% confidence intervals). The vertical line indicates the day of guppy introduction. Day 0 (*x*-axis) is as in Fig. 4.

stages of *Rivulus* reduces the impact of *Rivulus* on guppies and likely contributes to their ability to invade *Rivulus*-only streams.

Evidence for predation by guppies

Our laboratory experiments revealed that adult guppies of both sexes are capable of consuming early life stages of *Rivulus*. In addition to readily consuming free-swimming larvae (Fig. 1), guppies would attack late-stage *Rivulus* eggs (i.e., with dark, active embryos), break the chorion, and consume the embryo. However, we never observed an instance of predation on eggs at earlier stages of development. The mesocosm and channel experiments supported the hypothesis that guppies significantly increase the mortality of *Rivulus* larvae via predation. The marginally insignificant substrate × origin interaction in the mesocosms also suggests that complex substrates (e.g., abundant leafy debris) and ancestral experience with predators may mitigate the impact of guppies on naïve *Rivulus*, and in turn affect the rate of guppy invasion in streams previously inhabited only by *Rivulus*.

The decline in the abundance of juvenile *Rivulus* in each of the field introductions relative to their controls (Figs. 3 and 4) gives especially compelling evidence that guppies exerted a negative effect on juvenile *Rivulus*. Our finding of relatively few small individuals in the size distributions of both introductions contrasts with the expectation of more small individuals in populations experiencing resource limitation in the absence of predation (e.g., Nikolskii 1963, Persson 1988). The short-term reduction in the relative abundance of small *Rivulus* is consistent with guppies suppressing the production and/or survival of juvenile *Rivulus* to adult size.

The decline in juvenile abundance in the Introduction reaches also was reflected in increases in average size and declines in adult population densities through the first year post-introduction (Figs. 4 and 5). Notably, the rate and timing of divergence between the Introduction and Control reaches closely mirrors the rate and timing of growth in each stream's guppy population. While the guppy population in the Caigual Stream rose steadily throughout the first year after the introduction, the Taylor Stream population was nearly stagnant until Oct 2009 and grew rapidly thereafter (D. Reznick, *personal communication*). Thus, the time lag in the *Rivulus* population response to guppies (Figs. 4 and 5) likely reflects an inverse relationship between the guppy population density and juvenile *Rivulus* survival, consistent with a hypothesis of guppy predation. If the suppression of recruitment in *Rivulus* in these streams continues, it could lead to a long-term reduction in *Rivulus* density comparable to that seen in previous surveys of field sites by Gilliam et al. (1993).

Although competition and indirect effects also can increase juvenile mortality (e.g., by reducing food resources and growth rate, thus extending the time individuals are in a size range susceptible to size-dependent mortality; Werner and Gilliam 1984), the data presented here and in the literature point toward direct predation being the principal mechanism of guppy impact on *Rivulus* populations. Although the competition experiments revealed a significant negative impact on the growth of each species on the other, they also indicated that, under the conditions of the mesocosms, *Rivulus* undergo somatic growth at resource levels that cause guppies to lose mass. As a result, our data indicate that if the only interaction were resource competition, *Rivulus* would be able to exclude guppies and repel invasions (Table 1). Moreover, *Rivulus* grow larger than guppies and shift their diets toward terrestrial prey that are too large for guppies to consume (Fraser et al. 1999), potentially giving *Rivulus* a size escape from interspecific competition not accounted for in Eq. 3. Finally, *Rivulus* life histories in reaches with and without guppies are inconsistent with resource limitation as the guppy mode of impact on *Rivulus* evolution (Walsh and Reznick 2011). An indirect effect could increase juvenile mortality (e.g., guppies facilitate a pathogen that reduces *Rivulus* survival), causing the patterns observed in life-history studies. However, our laboratory demonstration of guppy predation on *Rivulus*, along with the consistency of the results across multiple experimental and field conditions, make direct predation the likely mechanism.

Complex interactions due to size structure

Traditional models of predation predict a negative effect on the prey population and a positive effect on the predator population (e.g., Berryman 1992); in contrast, size-structured mutual predation may benefit either species, depending on their relative success as both predator and prey (e.g., Wilbur 1988). Moreover, the *Rivulus*–guppy interaction is further complicated by their competition for food resources. Theory indicates that mutual intraguild predation destabilizes coexistence unless the species are well-segregated in space or time (van der Hammen et al. 2010). *Rivulus* often colonize habitats that are inaccessible or inhospitable to guppies, such as side pools, riffles, and seeps (Gilliam and Fraser 2001). Conversely, when the species co-occur in a stream reach, guppies are most prevalent in deep, open pools. Given the apparent threat that each species poses to the other, this spatial segregation may provide a refuge for juveniles of each species. Emigration of recruits from refugia to habitats dominated by the other species may be an important mechanism facilitating the coexistence of these mutual predators (D. F. Fraser, *unpublished data*).

Predation by guppies on *Rivulus*, predation by *Rivulus* on guppies (Seghers 1974, Endler 1983), and resource competition (Table 1) yields at least four distinct interspecific interactions between these species. Experimental manipulations of a community of two poeciliids

and a rivuliid, all with similar adult size, provide an interesting comparison (Taylor et al. 2001). In contrast to the bidirectional competition and predation in our system, Taylor et al. (2001) recognized only two strong interspecific interactions, both unidirectional. The greater complexity of interspecific interactions in our two-species system emphasizes the role of size structure in trophic interactions and community dynamics (de Roos and Persson 2001).

Viewed through the lens of niche theory, these results indicate that guppies not only reduce the realized niche of *Rivulus* via competition, intimidation, and/or direct mortality, but they also expand their own niche space. By reducing *Rivulus* density (Fig. 5) (Gilliam et al. 1993), guppies diminish the impact of a potential predator and strong competitor (Table 1). As a result, the size-dependent mutual predation described here should not only facilitate guppy invasions into habitats where *Rivulus* had been the only fish present, it should also strongly influence co-evolution of both species.

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SUPPLEMENTAL MATERIAL

Appendix

Summary statistics for each species combination used in the competition assays (*Ecological Archives* E094-056-A1).