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The comparative ecology of a killifish (*Rivulus hartii*) across aquatic communities differing in predation intensity

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ABSTRACT

Background: Life-history theory predicts that populations experiencing different patterns of age- or size-specific mortality will evolve divergent life histories. Higher mortality can also cause indirect effects by reducing population density and increasing resources for survivors. How life histories evolve can ultimately be shaped by the interactions between the direct impact of predators on mortality, their indirect effects on resource availability, and the age specificity of these combined effects. Prior research on the killifish, *Rivulus hartii*, suggests differences among aquatic communities in both predation risk and resource availability but has failed to characterize the age specificity of these effects.

Study organism and site: We studied *Rivulus hartii* in Ramdeen stream, a second-order tributary of the Arima River, on the south slope of the Northern Range Mountains in Trinidad. We used four sites near each other: in the two *Rivulus*-only sites, killifish were found alone; in the *Rivulus*/guppy site, guppies (a potential predator of juvenile *Rivulus*) also occurred; and in the high-predation site, *Rivulus* occurred with predators.

Hypotheses: (1) If guppies shape the evolution of *Rivulus* life histories by increasing juvenile mortality rates and indirectly increasing food availability to the survivors, then juvenile mortality and growth rates should be higher when guppies are present than when they are absent. (2) If larger predators shape *Rivulus* life histories by selectively preying on adult *Rivulus*, then the added mortality associated with predators should be greater in the larger size classes.

Methods: We performed mark–recapture studies on juvenile and adult *Rivulus* in the field (>12 mm total length), which allowed us to estimate size-specific mortality, growth rate, and density.

Results: *Rivulus* experienced the highest mortality in the high predation site, but predation was not selectively focused on adults. Furthermore, the higher mortality was coupled with reduced population density and increased adult growth rates. In *Rivulus*/guppy and *Rivulus*-only sites, all size classes had the same survival rate. Laboratory study confirmed that adult guppies can prey upon hatchling *Rivulus* (<7 mm) and are restricted to preying on this size class. Lastly, juvenile *Rivulus* from *Rivulus*/guppy localities had higher growth rates than those from *Rivulus*-only localities, as predicted, but such an effect disappeared in the adult stage.

Keywords: density, growth rate, guppy, indirect effects, killifish, life history, mark–recapture, mortality, *Poecilia reticulata*, *Rivulus hartii*.

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INTRODUCTION

The empirical study of life-history evolution has advanced in concert with the development of more sophisticated and realistic theoretical models (Stearns, 1992; Brommer, 2000; Roff, 2002). Early models considered the impact of age- or size-specific mortality rates in shaping life-history patterns (Gadgil and Bossert, 1970; Law, 1979; Charlesworth, 1980). The importance of mortality schedules in shaping life-history evolution has been demonstrated through laboratory experimental evolution (Stearns *et al.*, 2000). Such models also provide good approximations to observed life-history patterns in natural systems (Reznick and Endler, 1982). However, nature is inherently more complex than a laboratory environment. By focusing solely on mortality, these models implicitly ignored such factors as food availability, which can be affected by population density, and can in turn be regulated by predation (Wootton, 1994; Pace *et al.*, 1999). Adaptation, including life-history evolution, is shaped by multiple selective forces (Lack, 1954; Reznick and Travis, 1996; Grether *et al.*, 2001; Reznick *et al.*, 2001; Walsh and Reznick, 2009). Life-history models have added layers of complexity that incorporate such biological realism (reviewed in Brommer, 2000; Reznick *et al.*, 2002; Bassar *et al.*, 2010). In addition to age- or size-specific mortality rates, they have included the effects of resource levels (Stearns and Koella, 1986; Hutchings, 1993), density (Abrams, 1993; Abrams and Rowe, 1996), condition-dependence (Williams and Day, 2003), and the interaction of different mortality sources (Gardmark *et al.*, 2003). The development of such theory has expanded the scope of predictions, highlighting that, in addition to age- or size-specific mortality rates, a fuller understanding of life-history diversification in nature often requires the consideration of the ecological consequences of changes in mortality risk (Walsh, 2013).

One powerful way to test predictions of life-history theory in nature has been the comparative study of populations that differ in an environmental factor likely to select for opposing life-history strategies. Such an approach has been taken to evaluate the predicted response to mortality caused by predation (Reznick *et al.*, 1996; Johnson and Belk, 2001), season length (Tatar *et al.*, 1997; Dudycha and Tessier, 1999), and fluctuating resource levels (Bronikowski and Arnold, 1999; Sparkman *et al.*, 2007). Life-history predictions can be evaluated by characterizing the population biology and life-history characteristics of populations in these different environments. By rearing a subset of populations in a common environment, some investigators have assessed whether life-history divergence between populations has a genetic basis (Reznick and Bryga, 1996; Johnson, 2001; Morrison and Stacy, 2014). This has been the general framework for our research on life-history evolution in a Trinidadian killifish, *Rivulus hartii*.

The rivers of Trinidad's Northern Range Mountains often have waterfalls that truncate the upstream distribution of some fish species, creating natural variation in community composition. In the lower portions of rivers, *Rivulus* is found with several piscivorous species (high-predation localities). Above some barriers, *Rivulus* co-occurs only with guppies (*Poecilia reticulata*) (*Rivulus*/guppy localities). Further upstream, above additional barriers, *Rivulus* is the only fish species present (*Rivulus*-only localities).

We have documented consistent associations between *Rivulus* life-history phenotypes and the aquatic community across multiple river drainages (Walsh and Reznick, 2009; Furness *et al.*, 2011) in Trinidad's Northern Range Mountains. *Rivulus* from high-predation sites generally mature at a smaller size, produce more and smaller eggs, and allocate more resources to reproduction, as a proportion of total body weight. *Rivulus* from *Rivulus*-only sites are larger at maturity, produce fewer and larger eggs, and allocate less to reproduction. *Rivulus* from *Rivulus*/guppy sites show an intermediate life-history phenotype. Such differences in *Rivulus* life histories were found to remain after two generations of laboratory rearing on food levels chosen to approximate a growth rate seen in the field (Walsh and Reznick, 2008, 2010). Furthermore, the experimental introduction of guppies into what had previously been a *Rivulus*-only site caused the evolution of a life history typical of a *Rivulus*/guppy site (Walsh and Reznick, 2011).

Here we report the results of a mark–recapture experiment in high-predation, *Rivulus*/guppy, and *Rivulus*-only sites within the same river. We focus on sizes that range from 12 mm total length (the smallest size that could feasibly be marked) to the largest sizes represented in the population, and further study the interaction between larval *Rivulus* (<12 mm total length) and adult guppies in a laboratory setting. Our goal is to characterize the population biology of *Rivulus* in sites with differing predatory and competitor species, thus providing a link to our life-history studies. From prior work it is known that different predatory and competitive interactions occur in these three contrasting communities that alter *Rivulus* mortality rate, density, growth rate, population size-structure, habitat choice, and movement patterns (Fraser and Gilliam, 1987, 1992; Gilliam *et al.*, 1993; Fraser *et al.*, 1995, 1999, 2001, 2006; Gilliam and Fraser, 2001; Walsh *et al.*, 2011; Fraser and Lamphere, 2013), yet there exists several key gaps in our knowledge. Prior studies of *Rivulus* population biology did not include individuals <30 mm and hence did not characterize the comparative fates of juveniles in these different environments. They also did not include formal mark–recapture estimates for mortality in high-predation communities. We predict that *Rivulus hartii* will experience the highest adult mortality rates when predatory species are present and that adult *Rivulus* from *Rivulus*-only and *Rivulus*/guppy sites will not differ in mortality rate (Walsh *et al.*, 2011), since these sites lack predators capable of preying upon adult *Rivulus*. Furthermore, we predict that juvenile *Rivulus* from *Rivulus*/guppy sites will have lower survival but higher growth rates than juvenile *Rivulus* from *Rivulus*-only sites, owing to the direct and indirect effects of predation by adult guppies.

MATERIALS AND METHODS

Study site

The present study was conducted in Ramdeen stream, a second-order tributary of the Arima River, located on the south slope of the Northern Range Mountains in Trinidad. Natural barriers in this stream resulted in their being high-predation, *Rivulus*/guppy, and *Rivulus*-only localities in close proximity to one another. Our high-predation site contained *Hoplias malabaricus*, *Aequidens pulcher*, *Rhamdia quelen*, *Poecilia reticulata*, and *Synbranchus marmoratus*. There was a narrow side channel adjacent to the main stem of the tributary. *Rivulus* congregated at high density in the side channel and were virtually absent in the main river, where most predators were found. Our *Rivulus*/guppy site, approximately 100 m upstream, contained *Rivulus*, guppies, and *Synbranchus marmoratus*. In previous years, this site may have occasionally contained other fish species including the catfish *Rhamdia quelen* and *Hoplias malabaricus* (Fraser and Gilliam, 1992). However, these species were not observed in this site or in several pools immediately downstream during the four months of this study. The present study included extended nighttime fieldwork, which is when these species are active, so our not seeing them is a good indication that they were not present. Only *Rivulus* and *Synbranchus* are found further upstream; two such '*Rivulus*-only' sites from this region were chosen. *Synbranchus* will prey upon *Rivulus* in aquaria (A.F., personal observation) but these eels are uncommon in the upper Ramdeen and do not appear to impact *Rivulus* density (Fraser and Gilliam, 1992). Each of our 10–15 m long sites had a pool-riffle structure and was bounded by small waterfalls (*Rivulus*/guppy and two *Rivulus*-only sites). The short sampling reaches, bounded on either side by barriers to dispersal, facilitated a high recapture rate for even the small *Rivulus*. The proximity of these four sites to one another controlled for the potentially confounding effect of environment (see evolutionary-ecology.com/data/2916Appendix.pdf, Table S1).

Our mark–recapture study took place monthly over four consecutive months (September 2009 to early January 2010). *Rivulus* within a given site were captured with baited minnow traps and hand nets, and sites were visited during both daylight and night hours (approximately 2 hours search time per site per recapture). *Rivulus* were transported to the research station where all individuals were sexed, measured to the nearest hundredth of a millimetre, and weighed to the nearest thousandth of a gram. All *Rivulus* greater than 12 mm total length were given a combination of unique identifying marks via small subcutaneous injections of coloured elastomer (Northwest Marine Technology Inc., Shaw Island, WA). We used a two-mark code with four colours and seven body positions. *Rivulus* were returned to the location of capture no more than two days after capture. A small number of individuals lost one of their marks, but their identity could always be inferred from the remaining mark, sex, and size. Mortality during capture, marking, and release was minimal (22 of 1182 individuals).

In mark–recapture models, apparent survival (ϕ) is the probability the animal has survived and remained in the study area. Emigration thus inflates the estimated mortality rate (White and Burnham, 1999). During the last recapture, we collected *Rivulus* up- and downstream of all focal sites to determine the degree of emigration. The same effort was put into searching up- and downstream as was used to search the focal sites and all *Rivulus* encountered were captured and examined for marks. Few marked émigrés were found (2916Appendix.pdf, Table S2) and those that were marked were subsequently deleted from the data set. There was no emigration bias among sites (Table S2).

Size-specific survival

We assessed the effects of site (high-predation, *Rivulus*/guppy, and *Rivulus*-only 1 and 2), sex/stage (juvenile, male, female), and time (allowed to vary by month) on *Rivulus* apparent survival (ϕ) and recapture probability (p) while allowing for transitions from the juvenile stage to that of mature male or female (via the movement parameter: ψ). Our analyses were implemented using multi-state mark–recapture models (Nichols, 1992; Schwarz *et al.*, 1993; Nichols and Kendall, 1995) in the program MARK (White and Burnham, 1999).

Rivulus between 12 and 35 mm in total length were categorized as juveniles. *Rivulus* more than 35 mm in total length were categorized according to sex (male or female). This length roughly corresponds to the size when *Rivulus* reach sexual maturity (Walsh and Reznick, 2009; Furness *et al.*, 2011) and develop sex-specific markings; it thus represents a fairly biologically realistic switch-point at which to divide stage/sex classes. The probability that juvenile *Rivulus* transitioned to the adult size class (i.e. grew and crossed the 35-mm threshold) between recapture occasions was modelled by ψ, the movement parameter.

The fit of models was judged using QAIC$_c$ (Quasi-likelihood adjusted Akaike's Information Criterion) and following the selection recommendations of Burnham and Anderson (2002). Models with lower QAIC$_c$ values are a better fit to the data. When the difference in QAIC$_c$ is less than 2, the models being compared carry approximately equal support; when the difference is between 2 and 7, there is moderate support for a real difference between models; and when the difference is greater than 7, there is strong support for differences between models (Burnham and Anderson, 2002).

We began our model selection procedure by assessing and correcting for possible overdispersion in the data. We used the median-$\hat{c}$ test procedure with ϕ and p each parameterized with site × stage and ψ parameterized with site. This test estimates the variance inflation factor ($\hat{c}$) that can then be applied to subsequent model selection as a correction factor, resulting in the use of the $QAIC_c$. After 1200 simulations, the correction factor ($\hat{c}$) was estimated at 1.2336, indicating a relatively small amount of overdispersion. A $\hat{c}$ equal to 1 indicates perfect model fit, and applying corrections provided $\hat{c}$ is less than 3 is considered acceptable (Lebreton *et al.*, 1992, pp. 84–85).

We were particularly interested in determining whether survival probability differed among sites and varied as a function of sex and/or stage (juvenile vs. adult). Survival and recapture estimates were allowed to vary as a function of site, sex (male/female), and stage (juvenile/adult). In our model notation, which follows the standard convention, the × symbol between two factors indicates a full interaction, that is, the effect of one factor is estimated at all levels of the other factor (Lebreton *et al.*, 1992). In program MARK, corrections were made for the slightly uneven time intervals between sampling occasions.

Our high-predation site was sampled in October through January while the *Rivulus*/guppy and two *Rivulus*-only sites were sampled in September through December. We tested whether this offset in months had any discernible effects by comparing model selection results and parameter estimates when data for October, November, and December (the months when all sites overlap in the period of study) were included versus models that included data from all months, but did not explicitly include an effect of time. This latter approach implicitly assumes that all months are the same. Results were similar regardless of the method used, so only the analyses performed on the full data set are included.

We used a two-step approach to model selection. We first tested for an effect of sex on survival and recapture probability by comparing a fully parameterized model including this factor to models whose only difference was the absence of this factor for ϕ and p, respectively. Within sites there was overwhelming $QAIC_c$ support for no effect of sex on subsequent survival or recapture probability (2916Appendix.pdf, Table S3). All subsequent models were constrained such that within sites male and female (hereafter adult) survival was equal. In the second step, we created a list of 16 biologically realistic candidate models designed to test our specific hypotheses. We chose a candidate model set that included all combinations of site, stage (juvenile and adult), and their interaction for both probability of survival and recapture, while ignoring variation due to time. In each of these models, the probability that juvenile *Rivulus* transitioned to sexual maturity between recapture occasions (ψ) was allowed to vary by site. This assumption is reasonable because juvenile growth rate varied among sites (Table S3) and models that allowed this parameter to vary by site performed considerably better than models for which this factor was constant across sites (Table S3). We then turn to model-averaged survival estimates to more specifically address our original hypotheses. A model-averaging approach to the estimation of survival and recapture rates accounts for uncertainty in model selection by calculating a weighted average for each parameter estimate according to the degree of model support, as judged by $QAIC_c$ (Burnham and Anderson, 2004). By considering the relative support of each model in deriving parameter estimates, the model-averaging approach provides more robust estimates than any single model could (Johnson and Omland, 2004).

Growth rate

Rivulus growth rates were calculated as:

$$[(\ln(\text{weight}_{\text{recap}}) - \ln(\text{weight}_{\text{initial}}))/(\text{day}_{\text{recap}} - \text{day}_{\text{initial}})].$$

Each *Rivulus* recaptured on at least one occasion is represented as a single growth rate; when recaptured on multiple occasions, the growth rate was calculated between the first and last recapture occasion (i.e. over the longest interval). Growth rate data were also analysed when separate rates were calculated for each recapture period (such that a given individual could be represented by three separate growth rates, if it was recaptured on all four occasions). Both analyses yield similar results, so only that of the first described method is presented. Data from the two *Rivulus*-only sites were combined because there were relatively few juveniles in one of the *Rivulus*-only sites and a GLM analysis of adults revealed no significant differences between these sites and no significant site × length interaction. Models were fit separately for juvenile (Total length < 35 mm) and adult (Total length > 35 mm) *Rivulus*. Doing so amounts to a piecewise regression and adds biological realism to the analysis because 35 mm is the approximate divide between immature and mature individuals. At this chosen point, *Rivulus* also show a deceleration in growth. Our linear model for adult *Rivulus* growth rate included community (high-predation, *Rivulus*/guppy, and *Rivulus*-only) and sex (male, female) as fixed effects and total length as a covariate. All interactions were included and those that were not significant were sequentially deleted. A quadratic model was fit to juvenile growth rates. Community was entered as a fixed effect and length and length^2 were included as covariates. In the analyses of both juvenile and adult growth rates there was a significant interaction between community and initial length, indicating heterogeneity of slopes. Thus for significance testing, we specify the level of the covariate (Initial Length) at which the comparison is made. All statistical analyses were performed in PASW Statistics 18.

Abundance and size distribution

The number of *Rivulus*/m^2 and biomass/m^2 were estimated in each site using pools as the sampling unit (Gilliam *et al.*, 1993). Pools are defined as stretches of river where depth is above average and current is low. Using pools as the unit of comparison allows us to control for differences in habitat structure among sites. Pool area was estimated using the Sketch and Calc program (http://www.sketchandcalc.com/). The pool density estimates for our high-predation site include both the pool of the main channel, which was virtually void of *Rivulus* due to the presence of predatory *Hoplias*, and the narrow side channel, where *Rivulus* were abundant. Since each site was sampled monthly over four consecutive months, we were able to derive standard errors for our density estimates. *Rivulus* density differences among sites were compared using a Kruskal-Wallis test.

Rivulus population abundance was also estimated using the *POPAN* formulation (Schwarz *et al.*, 1993) of the Jolly-Seber class of models, implemented in MARK. This model allows for non-closure of the population due to recruitment within the study area. A separate abundance estimate was generated for each site at each recapture occasion, and these values were divided by the total water surface area of each site to calculate fish density, thus facilitating comparison among sites.

Laboratory predation trials

Rivulus hartii are 6–7 mm long at birth. The fate of size classes between this size and 12 mm remains unaccounted for by our mark–recapture study. We performed a series of laboratory predation trials ($n = 24$) to determine whether Trinidadian guppies prey upon these smaller size classes of *Rivulus hartii*. Each trial involved one mature female guppy (20–27 mm standard length) and 10 *Rivulus* of a given size class (either <7 mm or 7–9 mm total length) being placed in 10-gallon (45.46-litre) aquaria for 24 hours. Prior to the trial, guppies were maintained in stock tanks and fed baby brine shrimp or Tetramin flakes. Guppies and *Rivulus* were allowed to acclimate to trial tanks overnight while remaining separated; no food was added to the trial tanks, ensuring that neither *Rivulus* nor guppies had eaten for at least 12 hours prior to a trial. After the 24-hour trial, tanks were intensively searched and all remaining *Rivulus* captured and counted. The trial tanks contained only an air stone and a broken pot shard (to serve as bottom cover), thus ensuring that all surviving *Rivulus* were captured. The *Rivulus* and guppies used in the predation trials were F1 descendants of fish collected from a *Rivulus*/guppy tributary of the Aripo River, Trinidad. Individual guppies and *Rivulus* were used in only a single trial.

RESULTS

Survival and recapture probability

Rivulus survival and recapture probability varied among sites – the top three models all included an effect of site for both ϕ and p, and the ΔQAIC_c between the best model and the next best supported model not including an effect of site was 4.87, indicating a moderately high degree of support for there being differences among models (Table 1). There were different patterns of juvenile and adult survival among predator communities (site × stage).

The best model of our candidate set estimated survival as a function of site × stage and recapture as a function of site. The ΔQAIC_c between this model and the model with the second most support was 2.63, indicating a moderate degree of support for their being a real difference between models. Exploring the model-averaged parameter estimates allows us to specifically address our hypotheses. Both juvenile and adult *Rivulus* from high-predation sites showed lower survival rates than juvenile and adult *Rivulus* from *Rivulus*/guppy and *Rivulus*-only sites (Fig. 1, Table 2). There was no difference in adult survival between *Rivulus*-only and *Rivulus*/guppy sites. These results are consistent with our predictions. Contrary to expectation, juvenile survival in *Rivulus*/guppy sites was equivalent to that in the two *Rivulus*-only sites (Fig. 1). In two sites (high-predation and one of the *Rivulus*-only sites), adult survival was higher than juvenile survival. Monthly recapture rates varied as a function of site but were generally quite high, ranging from 0.74 to 0.95 for juveniles and 0.75 to 0.94 for adults (Table 2).

Growth rate

Juvenile and adult *Rivulus* displayed qualitatively different growth trajectories (Fig. 2), justifying the use of different models for these life stages. Juvenile growth rate was fit with a quadratic model. Juvenile *Rivulus* from the *Rivulus*/guppy site had higher early growth rates than juvenile *Rivulus* from the combined *Rivulus*-only sites, but these differences in growth

Table 1. Model selection results from mark–recapture analyses

Model structure							
Survival (ϕ)	Recapture (*p*)	Movement (ψ)	Parameters	QAIC$_c$	ΔQAIC$_c$	QAIC$_c$ weight	QAIC$_c$ deviance
Site × Stage	Site	Site	16	1161.37	0.00	0.5975	201.29
Site	Site	Site	12	1164.00	2.63	0.1603	212.20
Site	Site × Stage	Site	16	1165.07	3.69	0.0942	204.99
Stage	Site	Site	10	1166.24	4.87	0.0523	218.55
No effect	Site × Stage	Site	13	1167.69	6.32	0.0254	213.82
Site × Stage	Site × Stage	Site	20	1167.76	6.39	0.0245	199.33
No effect	Site	Site	9	1168.01	6.64	0.0216	222.37
Stage	Site × Stage	Site	14	1168.99	7.62	0.0133	213.06
Site × Stage	No effect	Site	13	1171.29	9.92	0.0042	217.43
Site × Stage	Stage	Site	14	1172.56	11.19	0.0022	216.63
Site	Stage	Site	10	1172.60	11.23	0.0022	224.91
Site	No effect	Site	9	1173.97	12.60	0.0011	228.33
Sage	No effect	Site	7	1175.43	14.06	0.0005	233.87
Stage	Stage	Site	8	1176.42	15.05	0.0003	232.82
No effect	Stage	Site	7	1176.70	15.33	0.0003	235.14
No effect	No effect	Site	6	1178.04	16.67	0.0001	238.51

Note: Site refers to high-predation, *Rivulus*/guppy, and *Rivulus*-only 1 and 2. Stage refers to juvenile versus adult.

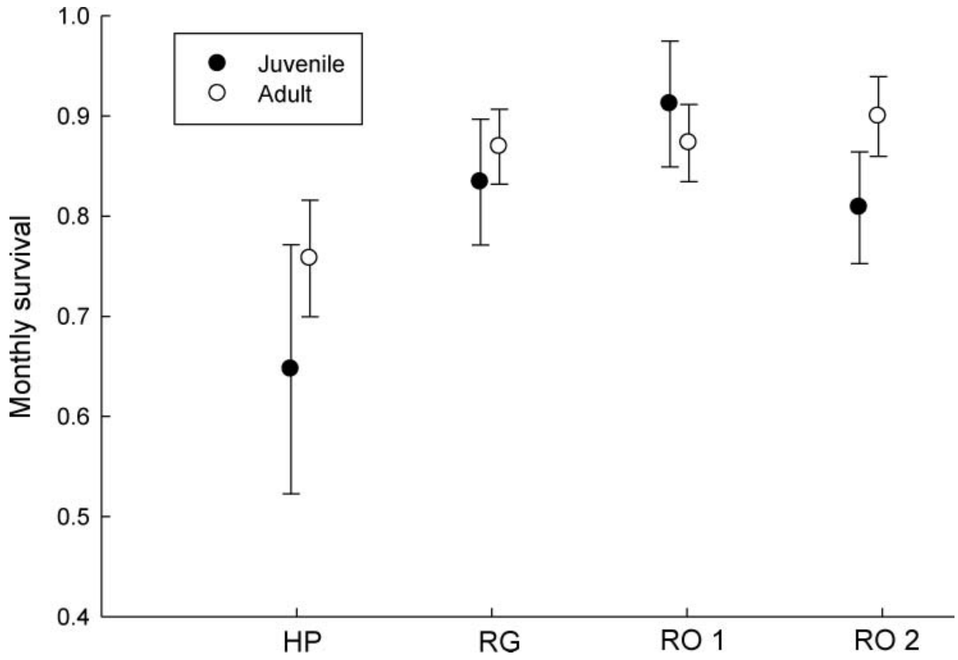

Fig. 1. Model-averaged survival estimates (mean and standard error) for juvenile and adult *Rivulus hartii* in high-predation (HP), *Rivulus*/guppy (RG), and *Rivulus*-only (RO 1 and 2) sites.

Table 2. Model-averaged parameter estimates for monthly survival, recapture probability, and probability of transitioning from juvenile to adult (ψ) in the four sites (standard errors in parentheses)

Site		Apparent survival (ϕ)	Recapture probability (p)	Transition (ψ)
High-predation	Juvenile	0.65 (0.124)	0.86 (0.131)	0.14 (0.056)
	Adult	0.76 (0.058)	0.91 (0.050)	
Rivulus/guppy	Juvenile	0.83 (0.063)	0.74 (0.057)	0.03 (0.017)
	Adult	0.87 (0.038)	0.75 (0.047)	
Rivulus-only 1	Juvenile	0.91 (0.063)	0.95 (0.038)	0.10 (0.036)
	Adult	0.87 (0.039)	0.94 (0.033)	
Rivulus-only 2	Juvenile	0.81 (0.056)	0.78 (0.048)	0.03 (0.012)
	Adult	0.90 (0.040)	0.80 (0.036)	

Table 3. Analyses of *Rivulus* growth rate

	d.f.	*F*-value
Juveniles		
Covariate:		
Length	1	17.321***
Length^2	1	4.187*
Main effects:		
Community	2	3.160*
Community × Length	2	4.689*
Error MS (d.f.)	2.5×10^{-5} (117)	
Adults		
Covariate:		
Length	1	11.325***
Main effects:		
Community	2	86.587***
Sex	1	10.198**
Community × Length	2	6.226**
Error MS (d.f.)	3.1×10^{-6} (206)	

Note: A quadratic model was fit to Juvenile growth data and a linear model to Adult growth data. Models initially included all interaction terms and those that were non-significant were sequentially deleted. In the analyses of both juvenile and adult growth rates there was a significant interaction between Community and Initial Length, thus for significance testing we specify the level of the covariate (Initial Length) at which the comparison of different communities is being made. In the *F*-tests above, the effect of Community was evaluated at the estimated marginal mean covariate level (22.23 mm for juveniles and 49.04 for adults).
Significance: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

disappeared as they approached maturity (Fig. 2, Table 3). Adult *Rivulus* from the high-predation site exhibited significantly higher growth rates than *Rivulus* from *Rivulus*-only and *Rivulus*/guppy sites. There were no differences in growth rate between adult *Rivulus* from *Rivulus*-only and *Rivulus*/guppy sites. Females exhibited a significantly higher growth rate than males, and this trend was apparent across all sites (Fig. 2).

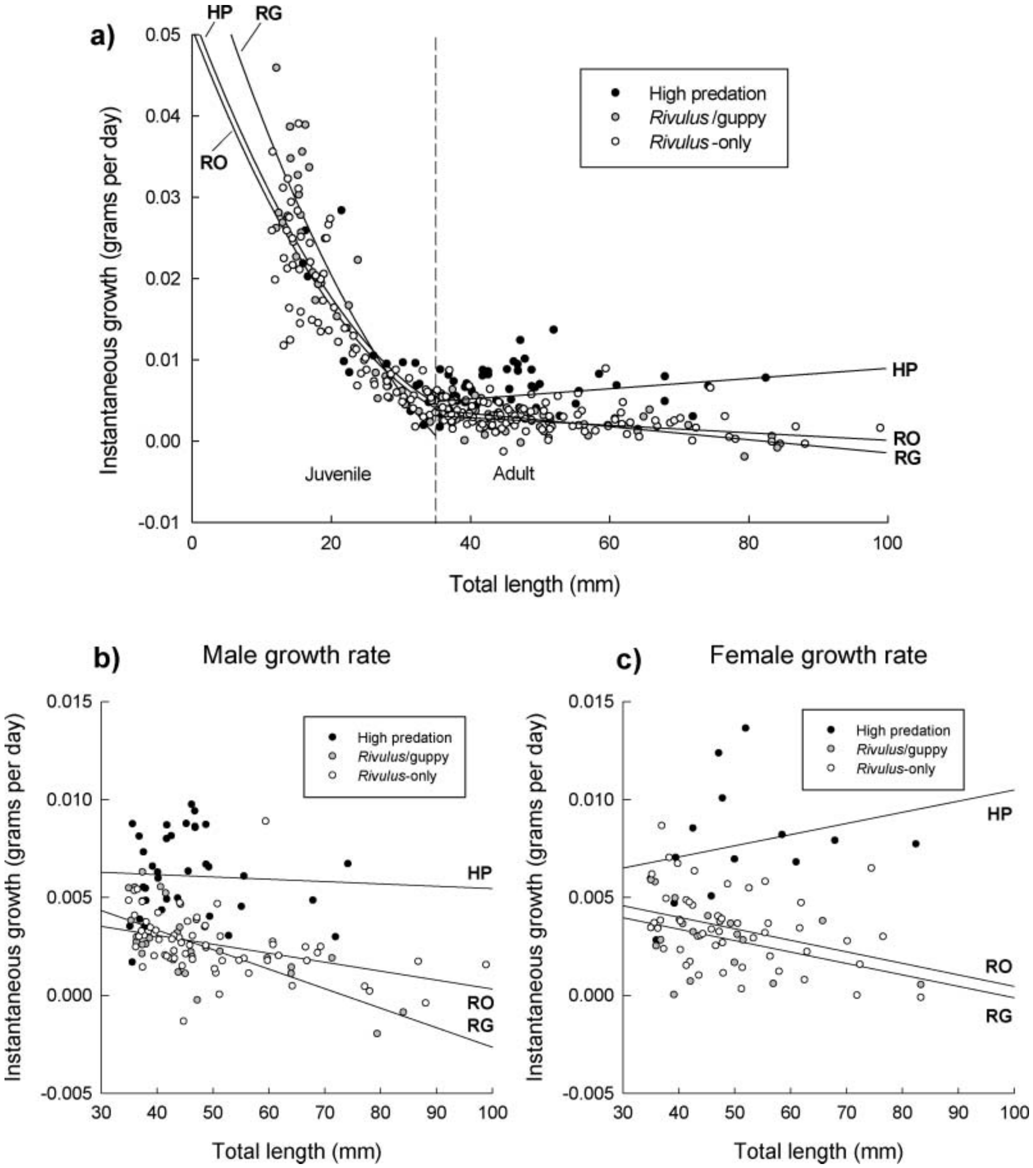

Fig. 2. (a) Instantaneous growth rate of juvenile and adult *Rivulus* across community types. A quadratic model was fit to juvenile growth rate and a linear model to adult growth rate. The dashed line is at 35 mm, the approximate point at which *Rivulus* reach sexual maturity. (b) Male and (c) female instantaneous growth rates (g/day) in high-predation, *Rivulus*/guppy, and *Rivulus*-only communities.

Abundance and size distribution

Using number of fish per m^2 as the estimate of density, there were significant differences among sites (Kruskal-Wallis: $H_{3,15} = 12.9$, $P = 0.005$). As expected, *Rivulus* density was lowest in the high-predation site, intermediate in the *Rivulus*/guppy site, and highest in the two *Rivulus*-only sites (Table 4). The two *Rivulus*-only sites differed in density, with the more upstream site having higher densities. This site had more vegetation surrounding and overhanging the stream. *Rivulus* in the high-predation site exhibited a spatial structure that minimized their exposure to the threat of predation. They avoided the main pool where *Hoplias* were found. *Rivulus* in *Rivulus*/guppy and *Rivulus*-only sites were found in all sections of river. Juvenile *Rivulus* were most abundant in the *Rivulus*/guppy and most upstream *Rivulus*-only site (Fig. 3). These sites had the most complex stream structure with several shallow low current areas where leaves and other detritus had accumulated, suggesting that these areas are favourable for *Rivulus* recruitment and juvenile survival (Fraser and Lamphere, 2013). Furthermore, these two sites had slightly lower recapture rates (Table 2), likely due to the abundance of potential hiding places.

Laboratory predation trials

Newly hatched *Rivulus* measured 6.37 ± 0.07 (S.E.) mm in total length. Adult female guppies preyed only upon newly hatched *Rivulus* less than 7 mm in total length (2916Appendix.pdf, Figs. S3, S4). The proportion surviving *Rivulus* in the < 7 mm category was 0.5 ± 0.12 versus 1.0 ± 0.0 (S.E.) in the 7–9 mm category (Mann-Whitney $U = 126$, $Z = 3.589$, $P < 0.001$).

DISCUSSION

Our goal was to characterize *Rivulus* size-specific mortality rate, size-specific growth rate, population density, and size distribution, thereby connecting our studies of *Rivulus* life-history evolution to the population biology of *Rivulus* in sites with different predator communities. Prior studies did not include formal mark–recapture data from high-predation communities or from juveniles. Inclusion of the smallest size classes is essential

Table 4. *Rivulus* density measured as number of individuals captured per m^2 (divided into adult and all size classes) and biomass per m^2 with river pools as the sampling unit (standard errors in parentheses)

	Pools as sampling unit			POPA formulation
	Rivulus per unit area			
	> 35 mm per m^2	All size per m^2	Biomass (g/m^2)	All size per m^2
High-predation	4.6 (0.3)	5.8 (0.6)	5.9 (0.8)	5.9 (0.4)
Rivulus/guppy	7.1 (0.5)	11.1 (1.7)	11.0 (0.9)	8.3 (0.5)
Rivulus-only 1	8.6 (0.4)	12.4 (0.5)	15.2 (1.6)	12.4 (0.4)
Rivulus-only 2	14.0 (0.9)	21.1 (1.8)	18.8 (1.3)	21.4 (0.6)

Note: Density of *Rivulus* was also estimated in each site using the POPAN formulation, which takes into account differences in probability of recapture among sites. In this formulation, density was estimated across total water surface area of the site (both pools and riffles).

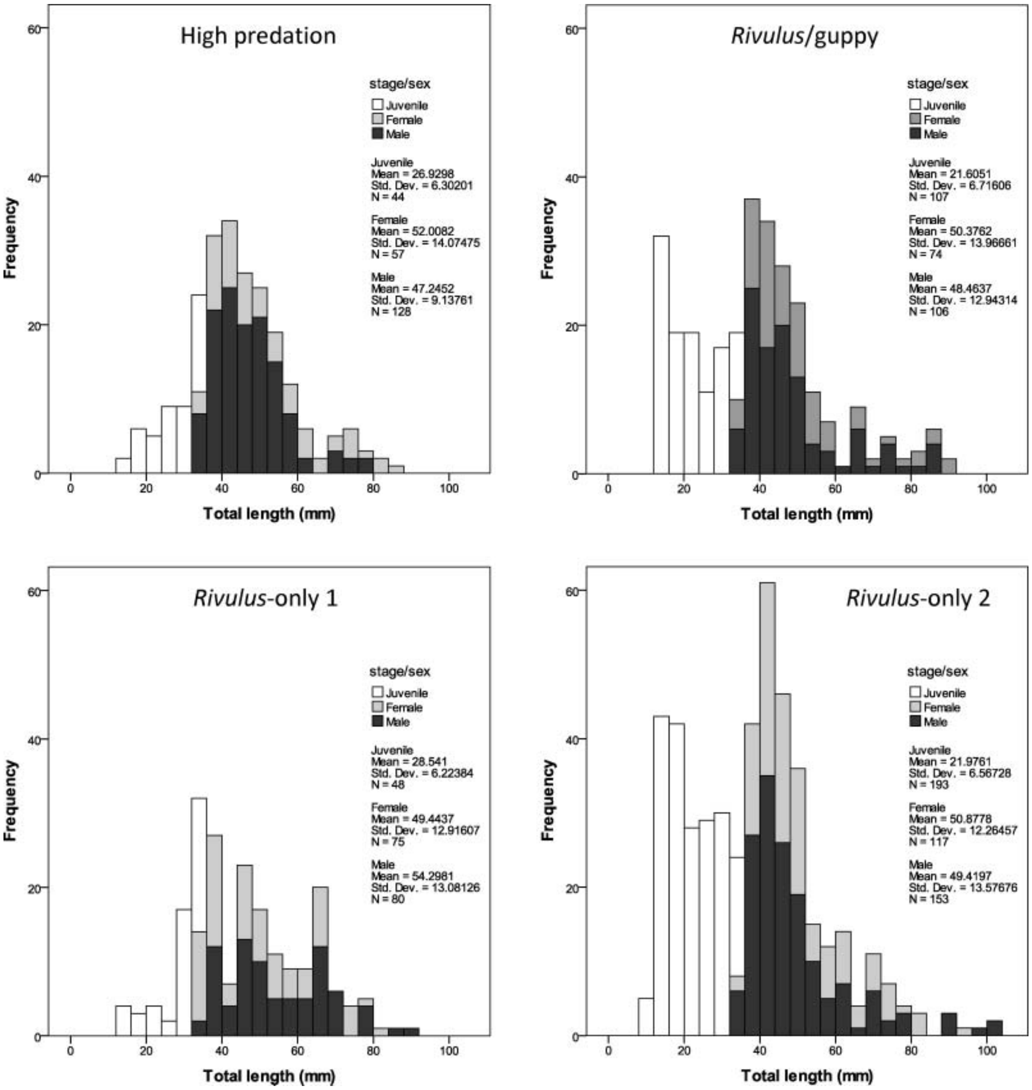

Fig. 3. Size frequency distribution of *Rivulus* in each site. Data from all recapture occasions were pooled and individuals were categorized as juvenile, male, or female.

for a complete assessment of the mechanism by which predators, including guppies, alter the population biology of *Rivulus*. Fraser and Lamphere (2013) used experiments in artificial streams and a field experiment in which guppies were introduced to previously guppy-free streams, to show that guppies depress *Rivulus* recruitment, reduce their population densities, and lead to a shift in the size distribution of *Rivulus* towards larger size classes. We expand on this work with our comparative study of survival and growth in three types of communities using an individual-based mark–recapture methodology. Below we discuss the implications of our results for *Rivulus* life-history evolution.

Adult *Rivulus* from the high-predation site exhibited lower monthly survival than *Rivulus* in *Rivulus*/guppy and *Rivulus*-only sites. This result is consistent with predation as a cause of increased mortality. Juvenile survival was also reduced in the high-predation site (relative to the other sites), suggesting that predators are not selectively targeting larger (mature) *Rivulus*.

Some life-history models considering age- or size-specific mortality predict that such a uniform reduction in survival will leave life histories unchanged (Gadgil and Bossert, 1970; Taylor *et al.*, 1974; Law, 1979). Such models use *r*, the intrinsic rate of increase, as the fitness measure and hence implicitly assume an absence of density regulation. However, if density dependence regulates populations, then such uniform mortality changes are expected to cause evolution of the life history (Kozlowski and Uchmanski, 1987; McNamara *et al.*, 2004). More recent models incorporating both size-specific predation and its effects on prey density (and hence resources) can lead to a multitude of different predictions including the life-history patterns observed in high-predation *Rivulus* (Abrams and Rowe, 1996). The increased mortality in our high-predation site was coupled with a reduced population density and an increased adult growth rate, indicating that indirect effects of predation are important in this system. Similar patterns of mortality, density, and growth have been found in Trinidadian guppies from high-predation environments (Reznick *et al.*, 1996, 2001).

Like our predecessors (Gilliam *et al.*, 1993; Walsh *et al.*, 2011; Fraser and Lamphere, 2013), we found that *Rivulus* from our *Rivulus*/guppy site had lower population densities than sites in which they were alone. Why are *Rivulus* densities lower when guppies are present? Evidence indicates that guppies are restricting *Rivulus* recruitment to the adult stage (Walsh and Reznick, 2010; Fraser and Lamphere, 2013). However, in the present study, *Rivulus* longer than 12 mm exhibited the same mortality rate in both *Rivulus*/guppy and *Rivulus*-only sites, suggesting that if guppies are targeting *Rivulus* as prey items, they are doing so at size classes below 12 mm. The hypothesis that guppies can and will prey upon larval *Rivulus* was confirmed through laboratory predation trials. In the laboratory environment, it was also found that guppies did not prey upon slightly larger *Rivulus* in the 7–9 mm range (2916Appendix.pdf, Fig. S3). Since the guppies we used as predators were among the largest size classes present in natural populations, our results suggest that only the smallest *Rivulus* are susceptible to guppy predation. It is possible that guppies also increase the mortality of *Rivulus* in the 7–12 mm size range via competition, but their net effect on larger size classes appears to be dominated by their causing a bottleneck during the juvenile life stage, rather than any competitive interaction with larger size classes (see also Fraser and Lamphere, 2013).

Why do guppies cause *Rivulus* to evolve? Mortality targeted towards smaller size classes is expected to cause the evolution of delayed maturity and reduced reproductive effort according to models considering only size-specific mortality rate (Gadgil and Bossert, 1970; Law, 1979; Charlesworth, 1980). However, *Rivulus* from *Rivulus*/guppy localities exhibit earlier maturity and higher reproductive effort than fish from *Rivulus*-only sites, and this pattern was observed in multiple river drainages (Walsh and Reznick, 2009). In other words, this life-history phenotype is anomalous if the cause of the difference is solely increased juvenile mortality when guppies are present. It has been proposed that patterns of increased juvenile mortality coupled with increased resources could lead to the observed life-history patterns (Walsh and Reznick, 2010; Walsh *et al.*, 2011), as models that include the effects of increased resources, and hence faster growth rates, can favour the evolution of increased reproductive effort and earlier maturity (Stearns and Koella, 1986; Hutchings, 1993; Abrams and Rowe, 1996). Furthermore, the observed interaction between food availability and life-history phenotype observed in a common garden laboratory environment argues for the importance of resource levels, an indirect effect of size-specific predation, as an important agent of selection driving local adaptation in *Rivulus* (Walsh and Reznick, 2008, 2010).

In the present study, *Rivulus* 12–35 mm long from our *Rivulus*/guppy site had higher growth rates than similar size classes of *Rivulus* from *Rivulus*-only sites. This pattern is consistent with an increase in per capita food availability, perhaps because of reduced competition with other *Rivulus* owing to their lower density. However, this study differs from that of Walsh *et al.* (2011), in that growth rate differences were not evident in the larger size classes. One potential explanation for this unexpected result is the effects of seasonality on *Rivulus* growth rate. Two of the three *Rivulus* mark–recapture studies detailed in Walsh *et al.* (2011) took place during Trinidad's dry season, whereas the present study was conducted within the wet season. Terrestrial invertebrate input, which makes up a large component of the *Rivulus* diet, shows strong seasonality in Trinidadian streams (Owens, 2010). Since season affects the abundance of food items (terrestrial vs. benthic invertebrates) available to *Rivulus* (Owens, 2010), it is plausible that *Rivulus* growth rate may vary as a function of season. This seasonality effect could potentially overshadow any site-specific growth rate differences.

Rivulus from the high-predation site exhibited the lowest density and adults exhibited the highest growth rates. These results are consistent with the hypothesis that an increased mortality rate results in lower *Rivulus* densities, which increases resources for remaining survivors resulting in higher growth rates. In addition, the characteristics of the high-predation site may contribute to the incredibly high growth rate exhibited by adult *Rivulus*. The site was bordered by tall grass and flying insects were abundant. Adult *Rivulus* prey heavily upon terrestrial insects (Owens, 2010). In the other sites, such bordering vegetation was absent and flying insects appeared to be less abundant. Furthermore, it was strikingly evident that *Rivulus* employ behavioural adaptations that seek to insulate them from predation risk. Nearly all *Rivulus* were found crowded in a narrow side channel, in contrast to the more widespread microhabitat used in *Rivulus*/guppy and *Rivulus*-only sites.

This study illustrates how the interaction of predation, population density of prey, resource availability, and the age specificity of these factors play out in a natural environment. The way life histories have evolved can only be explained if all of these factors are taken into account. These lessons are likely applicable to systems in which there is heterogeneity among age classes in risk of mortality, which are predicted to indirectly effect population density and hence resource availability (Walsh, 2013).

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