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Environmental factors influencing adult sex ratio in Trinidadian guppies

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Abstract Sex ratios can influence mating behaviour, population dynamics and evolutionary trajectories; yet the causes of natural sex ratio variation are often uncertain. Although secondary (birth) sex ratios in guppies (*Poecilia reticulata*) are typically 1:1, we recorded female-biased tertiary (adult) sex ratios in about half of our 48 samples and male-biased sex ratios in none of them. This pattern implies that some populations experience male-biased mortality, perhaps owing to variation in predation or resource limitation. We assessed the effects of predation and/or interspecific resource competition (intraguild predation) by measuring the local catch-per-unit-effort (CPUE) of species (*Rivulus* killifish and *Macrobrachium* prawns) that may differentially prey on male guppies. We assessed the effects of resource levels by measuring canopy openness and algal biomass (chlorophyll *a* concentration). We found that guppy sex ratios were increasingly female-biased with increasing CPUE of *Macrobrachium*, and perhaps also *Rivulus*, and with decreasing canopy openness. We also found an interaction between predators and resource levels in that the effect of canopy openness was greatest when *Macrobrachium* CPUE was highest. Our study thus also reveals the value of simultaneously testing multiple environmental factors that may drive tertiary sex ratio variation.

Keywords Life history · Male-biased sex ratio · Predation · Resource limitation · Sex-biased mortality

Introduction

Sex ratios can influence many individual- and population-level processes (see e.g., Evans and Magurran 1999; Hazlett et al. 2005; Steifetten and Dale 2006). Sex ratio variation may arise at different stages in the life cycle, including fertilisation (primary sex ratio), birth (secondary sex ratio) and maturity (tertiary sex ratio). Our focus here is on tertiary (adult) sex ratio variation, which is observed in a wide variety of species (e.g., Hailey and Willemsen 2000; Pettersson et al. 2004; Donald 2007). There may be several causes of this variation. First, sex-specific differences in age-at-maturity or migration patterns tend to skew sex ratios in favour of the earlier-maturing sex (Lovich and Gibbons 1990; Girondot and Pieau 1993) or the sex that migrates later (Reynolds et al. 1986; Colwell and Oring 1988). Second, sex-specific differences in mortality may be caused by differential susceptibility to predators (Britton and Moser 1982; Hairston et al. 1983), low resource levels (Clutton-Brock et al. 1991; Mulvihill et al. 1992), inter- or intra-sexual aggression (Sherman and Morton 1984; Hailey and Willemsen 2000), or the costs of parental care (Donald 2007). And, of course, these and other factors may act in

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combination, making them difficult to disentangle without accounting for their possible covariance. Our goal in the present study is to jointly assess the role of several, potentially covarying, environmental factors that might influence tertiary sex ratio variation in natural populations of Trinidadian guppies (*Poecilia reticulata*).

Guppies are an interesting system for studying sex ratio variation because this variation has many potential influences on mating behaviour (Evans and Magurran 1999; Jirotkul 1999), sexual selection (Jirotkul 2000), and dispersal (Croft et al. 2003). In addition, although guppies have secondary sex ratios that are typically 1:1 (Haskins et al. 1961; Seghers 1973; Brown 1982; Pettersson et al. 2004; but see Geodakyan et al. 1967), in nature they often show biased tertiary sex ratios that can differ dramatically among populations (Haskins et al. 1961; Seghers 1973; Liley and Seghers 1975; Rodd and Reznick 1997; Pettersson et al. 2004). Moreover, there is evidence that several environmental factors may influence this variation. The leading candidates that have previously been found to have important effects on other guppy life history traits (e.g., Reznick 1982; Grether et al. 2001; Reznick et al. 2001) are predation and resource limitation.

Inferences regarding the role of predation often start from the observation that female biases are strongest at sites that lack severe predatory fishes (Seghers 1973; Liley and Seghers 1975; Rodd and Reznick 1997). These low-predation sites instead can have high densities of less severe guppy predators, such as killifish (*Rivulus hartii*) and freshwater prawns (*Macrobrachium* spp.), both of which attack guppies readily in the laboratory, but are generally considered mild predators in the wild (Magurran 2005). These weaker predators might still influence guppy sex ratio, however, because they may have some difficulty killing larger guppies, which are usually females (Reznick and Miles 1989). *Rivulus* has already been championed in this role, and laboratory studies have confirmed that smaller guppies are more vulnerable to *Rivulus* predation (Liley and Seghers 1975; Mattingly and Butler 1994). *Macrobrachium* might also exhibit size-biased, and therefore sex-biased, predation. As detailed below, however, since *Rivulus* and *Macrobrachium* likely prey on and compete for some of the same food resources as guppies, we will consider these species potential intraguild predators (Polis et al. 1989).

Sex ratio differences between high- and low-predation sites (Seghers 1973; Liley and Seghers 1975; Rodd and Reznick 1997) might also point to a role of resource limitation, because low-predation sites typically have lower primary productivity and higher competition. These sites are characterised by low productivity because they are found in small, low-order streams with more closed canopies that receive less light (Reznick et al. 2001). This reduces the availability of attached unicellular algae (Grether et al. 2001; Reznick et al. 2001), which is the primary food source for guppies (Dussault and Kramer 1981). Low-predation sites are characterised by high intra-specific competition because guppies are much more abundant and larger here than in the downstream, high-predation sections of the streams (Reznick et al. 2001). They are also characterized by higher inter-specific competition due to the higher *Rivulus* and *Macrobrachium* densities at these sites, as described above, since the diets of both *Rivulus* (Gilliam et al. 1993) and *Macrobrachium* (Covich and McDowell 1996) likely overlap with that of guppies (Dussault and Kramer 1981). All of the above effects may influence resource limitation for guppies, which may then contribute to male-biased mortality. The reason is that males devote less time to foraging and much more time to courtship than do females (Dussault and Kramer 1981; Magurran and Seghers 1994). Indeed, work on other poeciliid fishes suggests that males may be more susceptible than females to starvation (Schultz 1977).

Complicating attempts at simple inference based on natural variation, the effects of predators and resource levels may interact to influence guppy sex ratio. As one possibility, differences in guppy feeding behaviour under low resource levels (e.g., Kolluru and Grether 2005) might increase their predation risk. As another possibility, interspecific resource competition between guppies and *Rivulus*/*Macrobrachium* might increase disproportionately under low resource levels. Interactions between predators and resource levels have certainly been found to affect population demography (Krebs et al. 1995; Zanette et al. 2003) and life history evolution (Walsh and Reznick 2008) in other taxa. To our knowledge, however, studies of sex ratio variation in nature have not examined the potential interactive effects of predators and resource levels.

Sex ratio variation in guppies thus appears to be a problem with several potential solutions, none of which have been explicitly evaluated in nature or in relation to each other. This is our goal in the present paper. To this end, we estimated tertiary sex ratio, *Rivulus* and *Macrobrachium* abundance, and resource levels in natural guppy populations in Trinidad, and evaluated the relative effects of these factors and interactions in formal model comparisons.

Materials and methods

Study sites

We sampled 28 sites in the Marianne and Paria watersheds on the north slope of Trinidad's Northern Range Mountains [see Fig. S1 in electronic supplementary material (ESM)]. Sampling took place during the dry season at 25 sites in

March of 2006 and 23 sites in March of 2007 (20 of these sites were sampled in both years; ESM, Table S2). Some of the sites were categorised as high-predation and others as low-predation (ESM, Fig. S1; Crispo et al. 2006; Hendry et al. 2006; Millar et al. 2006), but we focussed on the latter, because it is here that sex ratios were expected to vary most dramatically (Seghers 1973; Rodd and Reznick 1997). In selecting sites, we attempted to minimise covariance among environmental factors that might contribute to the demography and evolution of guppies. Consequently, the factors of most interest in the present study (*Rivulus/Macrobrachium* abundance and resource levels) proved not to be strongly correlated among our study sites (see below).

Sex ratio

At each of two to eight pools per site per year, we sampled guppies in one or two sets of 30 min. Each of these sets consisted of two people fishing the same pool for 15 min, or three people fishing the same pool for 10 min. Exceptions were made for very small pools, where a single person could catch all (or nearly all) of the guppies in only 15 min. In 2006, many different pools were sampled in two sets of 30 min each, and sex ratio did not differ on average between the first and second sets (paired *t*-test, $t_{37} = -0.03$, $P = 0.488$). This result convinced us that a single set per pool would be sufficient to obtain an unbiased sex ratio estimate. We therefore used only a single set per pool in 2007, unless a pool was so large that full sampling required two sets.

During a given set, each person used two butterfly nets to catch as many guppies as possible. The captured guppies were placed in pool- and set-specific 10 l buckets that were half full of water. We lightly anaesthetized these fish with tricaine methanesulfonate (MS-222) and sexed them based on external features (colouration, gonopodium, gravidity). All sexing was done unambiguously by one person (A.P.H.). Small guppies with no obvious sexual characteristics were considered immature and were excluded from analyses. We thus focussed on adult (tertiary) sex ratio, since juvenile sex ratio does not differ significantly from 1:1 in the wild (Pettersson et al. 2004). After processing, the fish were allowed to recover in buckets and were then released back into the pools from which they had been caught.

Rivulus and *Macrobrachium*

Although we were able to characterise our sites as low-predation or high-predation (ESM, Fig. S1), we (like other authors) did not quantify the abundance of major predatory fishes. These fishes are only found at the few high-predation sites, are only episodically present, and are not amenable to precise quantification. We instead focussed on the weaker predators (*Rivulus* and *Macrobrachium*), which can be found at both low- and high-predation sites. As noted earlier, these may also be competitors for food eaten by guppies (intraguild predators). In March of 2007, we used standard trapping methods (see below) to capture these predators at each of our study sites. The resulting capture rates likely reflect some combination of the effects of density, activity levels, and responses to the potential food items that we used as bait.

Our trapping protocol involved the deployment of silver Gee minnow traps (Cuba Specialty Manufacturing, Fillmore, NY), each baited with six pellets of dry dog food. The traps were spaced a minimum of 3 m apart in slow-to-medium current and where the water was at least 25 cm deep. At each site, we used between three and five traps depending on the number and size of pools that we had sampled for guppies. We deployed traps at all such pools, except for two where the water was too shallow (depth < 25 cm). Our findings do not change if sex ratio data from these two pools are removed from the analyses (results not shown). After deployment, the traps were left undisturbed for 40 min, because previous work showed that catches taper off after this length of time (N. Millar, personal communication). All captured *Rivulus* and *Macrobrachium* were then enumerated and released back into the pools from which they had been caught.

We enumerated all *Macrobrachium* prawns with obvious claws, because these are used to capture guppies (A.E.M. and A.P.H., personal observation). Owing to this criterion, and to our day-time sampling, most of the captured prawns were probably *Macrobrachium crenulatum* (Endler 1983). We then calculated catch-per-unit-effort (CPUE) based on the number of individuals caught in each trap, where one trap-hour is one unit of effort. These trap-specific CPUE estimates were then averaged across all the traps at a given site.

For *Rivulus*, we were not confident in the accuracy of CPUE estimates based on only three to five traps per site. The reason is that *Rivulus* is much less common than *Macrobrachium*, and is often found in areas where traps are not efficient, particularly very shallow water and small side-pools (Gilliam et al. 1993; A.E.M. and A.P.H., personal observation). For 2007, we therefore base our analysis on *Rivulus* presence/absence, as indicated by three methods: traps, inadvertent capture while fishing for guppies, and direct observation. In addition, we calculated *Rivulus* CPUE (as for *Macrobrachium*, see above) based on much more intensive trapping conducted in 2004 and 2005. In those years, 19 of our study sites were sampled multiple times (mean number of sampling periods per site = 2.74) and with more traps (mean number of traps per site/period = 8.62). Millar et al. (2006) conducted this sampling, but those authors did not calculate or report *Rivulus* CPUE. We found a strong correlation in *Rivulus* CPUE between 2004 and 2005 ($r = 0.64$, $n = 19$, $P = 0.006$) and a strong association between *Rivulus* presence/absence in 2007 and *Rivulus* CPUE in 2004–2005 (logistic regression: $R^2 = 0.19$, $n = 19$, $P = 0.032$). It is therefore reasonable to assume that *Rivulus* abundance is relatively consistent between years at these sites (although it can vary at other sites or times: Fraser and Gilliam 1992). However, given the fact that we did not have high quality CPUE estimates from the year of our sex ratio sampling, our results for *Rivulus* effects should be considered tentative.

For both *Rivulus* and *Macrobrachium*, captured individuals were categorised into two arbitrary size categories (large > 50 mm and small < 50 mm) based on total length for *Rivulus* and on the distance from the tip of the chelipeds to the end of the telson for *Macrobrachium*. All of the analyses described below were conducted for all captured individuals and for large individuals only. Results (not shown) did not differ between the two sets of analyses and so we report only the former.

Algal biomass and canopy openness

Resource levels relevant to guppies are related to algal biomass (Dussault and Kramer 1981; Grether et al. 2001). To quantify algal biomass, we used a modified brush-syringe Loeb (1981) sampler to scrape periphyton from five underwater rocks per pool at 22 of our sites in 2007. Selected rocks were those that showed intermediate algae growth compared to other rocks in the same pool (e.g., Grether et al. 2001). The collected material was transferred on ice from the field to the laboratory, filtered, and then frozen until analysis. Pigments were extracted in 95% ethanol for 24 h, and chlorophyll *a* concentration was determined via fluorometry. Chlorophyll *a* concentration is a direct measure of instantaneous algal biomass at any given time, but we were more interested in average algal levels over longer time periods. For this, researchers often use canopy openness, because this can be rapidly quantified with reasonable precision and often relates well to algal biomass (Grether et al. 2001).

We measured canopy openness at each pool at each of the 23 sites sampled in 2007. These measurements were taken by a single person (A.E.M.) using a concave spherical densitometer (Lemmon 1957), which generates openness estimates that correlate well with those obtained by hemispherical photography (Englund et al. 2000; Grether et al. 2001). While standing in the centre of each pool, four measurements were taken, one facing each cardinal direction. Site means were calculated for percent canopy openness. Across the 22 sites with both types of measurements, canopy openness was positively associated with chlorophyll *a* concentration ($R^2 = 0.36$, $n = 22$, $P = 0.003$). For the reasons described above, all subsequent analyses therefore used canopy openness rather than algal biomass.

Data analysis

We used JMP v. 5.1 (SAS Institute, Cary, NC, http://www.jmp.com/) and "R" v. 2.4.1 (R Foundation for Statistical Computing 2006, http://www.r-project.org) for all statistical analyses. Analyses were always based on site means, and sex ratio was always expressed as the proportion of males (number of males divided by the total number of adults). We first checked the data for normality. Only percent canopy openness was significantly non-normal, and so this variable was arcsine square root transformed prior to analysis. Next, we evaluated the possibility of multicollinearity by considering correlation coefficients among our environmental factors, as well as the variance inflation factor (VIF; Glantz and Slinker 2001) for each environmental variable in our statistical models.

Our statistical analyses first considered sex ratio biases at each site and consistencies in this bias between years. We performed binomial tests at each site in each year to examine deviations from sex ratio equality (Wilson and Hardy 2002). For $n > 35$ adult guppies, we used the normal approximation to the binomial, corrected for continuity (Siegel and Castellan 1988). We next used Pearson's correlation to test whether variation in sex ratio among sites was correlated between 2006 and 2007. All subsequent analyses were based on the 2007 (rather than 2006) samples because (1) sex ratio estimates were more precise in 2007 owing to the larger number of captured guppies (ESM, Table S2), and (2) predator and resource level data were also collected in 2007.

We then examined whether spatial variation in sex ratio was associated with environmental factors when each was considered individually. These analyses were based on *t*-tests [*Rivulus* presence/absence, predation regime (high or low), and watershed (Marianne or Paria)] and simple linear regressions weighted by sample size (*Macrobrachium* CPUE, *Rivulus* CPUE in 2004–2005, and canopy openness). All relevant analyses were then repeated for the low-predation sites only, so as to evaluate whether or not any trends were driven by the few high-predation sites. We also repeated the above analyses for each watershed separately.

Finally, we examined the effects of multiple environmental factors considered together by evaluating the fit of alternative models. These sought to explain sex ratio (weighted by sample size) as a function of *Macrobrachium* CPUE, *Rivulus* presence/absence in 2007, canopy openness, watershed, and their interactions. Alternative models were evaluated in an information-theoretic framework (Burnham and Anderson 2002) that compared generalised linear models with binomial errors and a logit link function (Wilson and Hardy 2002). The tested models included all possible combinations of the four factors (*Macrobrachium* CPUE, *Rivulus* presence/absence, canopy openness, and watershed) and the two interactions relevant to our hypotheses (canopy openness by *Macrobrachium* CPUE and canopy openness by *Rivulus* presence/absence). We also included a null model (i.e. a constant only). Model selection was based on the Akaike information criterion corrected for sample size (AICc) (Burnham and Anderson 2002). This analysis was also repeated after replacing *Rivulus* presence/absence with *Rivulus* CPUE in 2004–2005.

Results

Our analyses were not afflicted by multicollinearity. Correlation coefficients among variables were all <0.5 (Table 1; values >0.8 indicate possible multicollinearity; Glantz and Slinker 2001). Moreover, VIFs in models that did not contain interaction effects were all <2.0 (VIF > 10 represents evidence of significant multicollinearity, and VIF > 4 represents moderate multicollinearity; Glantz and Slinker 2001). VIFs increased markedly in models containing interaction effects (range: 1.1–53.6), but this is expected when models include multiplicative terms and should not influence interpretation (Friedrich 1982).

Significantly female-biased sex ratios were recorded at 14 of 25 sites (56%) in 2006 and at 13 of 23 sites (56.5%) in 2007 (Fig. 1; ESM, Table S2). No significant male-biased sex ratios were evident. After step-up sequential Bonferroni correction (Hochberg 1988), female biases remained significant at 52% of the sites in 2006 and at 43.5% of the sites in 2007. These corrected results are noteworthy given the overly conservative nature of Bonferroni corrections (Moran 2003). For sites sampled in both 2006 and 2007, a positive correlation was evident between years when weighted by sample size ($r = 0.45$, $n = 20$, $P = 0.005$).

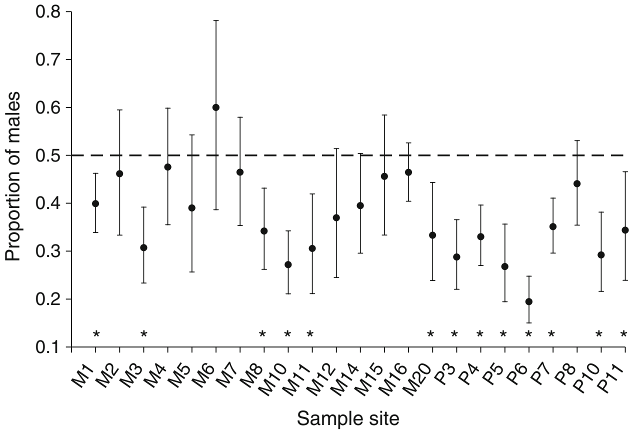

Fig. 1 Tertiary (adult) guppy (*Poecilia reticulata*) sex ratio (proportion of males ± 95% CI) at sites in the Marianne (*M*) and Paria (*P*) watersheds on the north slope of Trinidad's Northern Range Mountains sampled in 2007 (ESM, Fig. S1). The *dashed line* represents an equal sex ratio. Asterisks above the *x*-axis indicate significant deviations ($P < 0.05$) from equal sex ratio using the binomial test

Table 1 Correlation coefficients among environmental factors examined for associations with guppy sex ratio

	Factors	can	mac	riv
2007 data[a]	mac	−0.46*		
	riv	−0.16	0.34	
	wat	0.30	−0.38	−0.28
2007 data but *Rivulus* CPUE 2004–2005[b]	mac	−0.43		
	riv	−0.35	0.01	
	wat	0.24	−0.43	−0.20

can Canopy openness, *mac Macrobrachium* catch-per-unit-effort (CPUE), *riv Rivulus*, *wat* watershed (Marianne or Paria)

* Significant at $P < 0.05$

[a] $n = 23$ sites; *riv* = *Rivulus* presence/absence

[b] $n = 19$ sites, *riv* =*Rivulus* CPUE

Sex ratio was strongly associated with several environmental factors when each was considered individually. In particular, the proportion of males was lower at sites with higher *Macrobrachium* CPUE ($R^2 = 0.50$, $n = 23$, $P < 0.001$; Fig. 2a) and with less open canopies ($R^2 = 0.38$, $n = 23$, $P = 0.002$; Fig. 3). The proportion of males was not related to *Rivulus* presence/absence in 2007 ($t_{21} = -1.18$, $P = 0.252$), but was negatively associated with *Rivulus* CPUE in 2004–2005 ($R^2 = 0.27$, $n = 19$, $P = 0.022$; Fig. 2b). Sex ratio was associated with watershed: the Paria had a significantly lower proportion of males than did the Marianne ($t_{21} = 2.62$, $P = 0.02$). Sex ratio was not strongly associated with the low- versus high-predation contrast ($t_{21} = 1.69$, $P = 0.106$), although the trend was for a greater proportion of males in the latter (proportion males ± 95% CI: low-predation: 0.36 ± 0.04, high-predation: 0.43 ± 0.08). Note, however, that we sampled few high-predation sites and so we do not consider this a definitive test of the effects of predation regime.

All of the relevant statements above continue to hold if analyses are based on only the low-predation sites (*Macrobrachium* CPUE, $R^2 = 0.53$, $n = 18$, $P < 0.001$; canopy openness, $R^2 = 0.45$, $n = 18$, $P = 0.002$; *Rivulus* presence/absence in 2007, $t_{16} = -0.639$, $P = 0.532$), except that the effect of *Rivulus* CPUE in 2004–2005 becomes marginally non-significant ($R^2 = 0.21$, $n = 19$, $P = 0.089$). They also hold if only sites from the Marianne watershed are included in the analyses (*Macrobrachium* CPUE, $R^2 = 0.31$, $n = 15$, $P = 0.031$; canopy openness, $R^2 = 0.45$, $n = 15$, $P = 0.006$; *Rivulus* presence/absence in 2007, $t_{13} = -1.17$, $P = 0.261$;

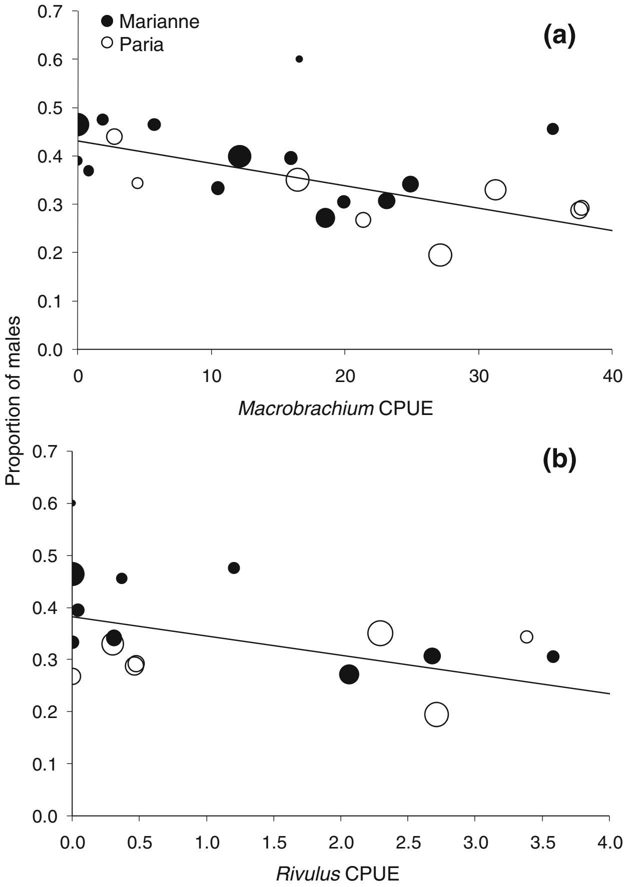

Fig. 2 Simple linear relationships for the Marianne and Paria watersheds between guppy sex ratio and **a** *Macrobrachium* catch-per-unit-effort (*CPUE*) in 2007, and **b** *Rivulus* CPUE in 2004–2005. Both regressions are weighted by sample size, as indicated with *circle* sizes

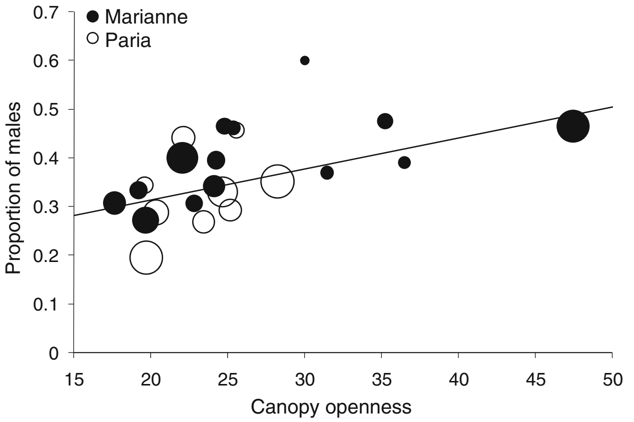

Fig. 3 Simple linear relationship for the Marianne and Paria watersheds between guppy sex ratio and canopy openness in 2007. The regression relationship is weighted by sample size, as indicated with *circle* sizes. Canopy openness is arcsine square root transformed

Rivulus CPUE in 2004–2005, $R^2 = 0.50$, $n = 15$, $P = 0.010$). The directions of these effects are also the same in the Paria, although statistical significance was lacking (*Macrobrachium* CPUE, $R^2 = 0.34$, $n = 8$, $P = 0.131$; canopy openness, $R^2 = 0.26$, $n = 8$, $P = 0.201$; *Rivulus* presence/absence 2007 $t_6 = -0.397$, $P = 0.705$; *Rivulus* CPUE in 2004–2005, $R^2 = 0.04$, $n = 7$, $P = 0.657$), probably due to the smaller sample size in the Paria.

The above findings were echoed and amplified in AIC model selection that jointly considered the effects of multiple environmental factors. When considering the 2007 data only, the best model included canopy openness, *Macrobrachium* CPUE, watershed, and the canopy openness by *Macrobrachium* CPUE interaction (Table 2). This model was 4.6 times more likely than the second best model, which included the above factors as well as *Rivulus* presence/absence, and it was 5.4 times more likely than the third best model, which included the above factors but without watershed. Results were similar when *Rivulus* presence/absence data were replaced with *Rivulus* CPUE in 2004–2005 data (Table 2). The importance of the watershed effect in our models suggests the merits of separate analyses within each watershed. We could not do this based on the present sampling because the number of terms in our models approached the sample size (number of sites) within the Marianne and exceeded it in the Paria. However, as noted above, analogous trends were found in both watersheds when each environmental factor was considered individually.

In summary, all analyses support the following inferences. First, the proportion of males decreases with increasing *Macrobrachium* CPUE and with decreasing canopy openness. Second, the effect of canopy openness is stronger when *Macrobrachium* CPUE is higher (interaction term, Fig. 4). Note, however, that this interaction needs to be interpreted with caution for two reasons: (1) certain combinations (high *Macrobrachium* and very open canopies) are very rare in nature and therefore also in our dataset, and (2) all of the sites with very open canopies (>25% openness) were from a single watershed (Marianne) (Fig. 4). In contrast to the above consistent results, only some analyses showed that the proportion of males decreased with increasing *Rivulus* CPUE.

Discussion

Although 1:1 secondary (birth) sex ratios are considered the norm for most vertebrate species (Cockburn et al. 2002), tertiary (adult) sex ratios often vary widely (e.g., Hailey and Willemsen 2000; Pettersson et al. 2004; Donald 2007). For instance, a recent review of sex ratios in over 100 wild bird populations found that secondary sex ratios did not generally differ from unity, whereas adult males tended to outnumber adult females (Donald 2007). Similarly, despite 1:1 secondary sex ratios (Haskins et al. 1961; Seghers 1973;

Table 2 Factors explaining variation in guppy sex ratio as informed by Akaike information criterion (AIC) model selection

	Model factors[a]							K	AICc	Δ_i	w_i
	Model rank	can	mac	riv	wat	can × mac	can × riv				
2007 data	1	+	+		+	+		5	151.21	0.00	0.65
	2	+	+	+	+	+		6	154.25	3.04	0.14
	3	+	+			+		4	154.68	3.46	0.12
	4	+	+	+		+		5	156.80	5.59	0.04
	5	+	+	+	+	+	+	7	158.42	7.21	0.02
2007 data but *Rivulus* CPUE 2004–2005	1	+	+		+	+		5	124.47	0.00	0.41
	2	+	+	+	+	+		6	125.79	1.32	0.21
	3	+		+	+		+	5	127.33	2.86	0.10
	4	+	+	+		+		5	127.99	3.52	0.07
	5	+	+	+	+		+	6	129.61	5.14	0.03

The number of estimable parameters (K), AIC corrected for sample size (AICc) values, AICc differences (Δ_i), and AICc weights (w_i) for the five most supported models are shown

[a] See Table 1 for abbreviations; factors included in a particular model have a "+" sign in that row

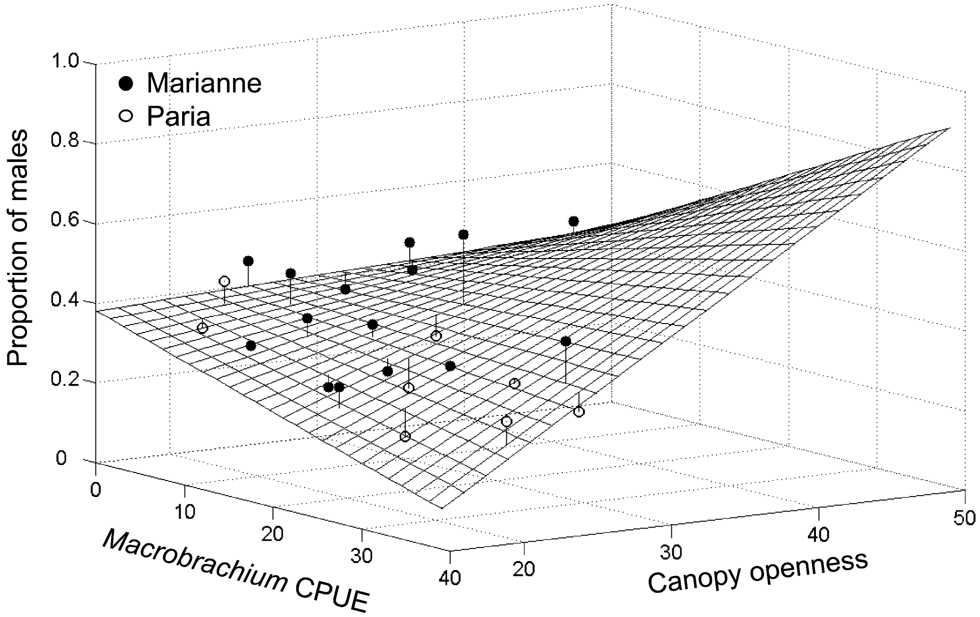

Fig. 4 Interactive effects of *Macrobrachium* CPUE and canopy openness on guppy sex ratio. The figure shows the predicted surface for the best model (includes canopy openness, *Macrobrachium* CPUE, watershed, and canopy openness by *Macrobrachium* CPUE; see Table 2) explaining guppy sex ratio variation as well as the actual data points for *Macrobrachium* CPUE, canopy openness, and sex ratio for the Marianne and Paria watersheds. The distribution of values on the main axes can be seen in Figs. 2a and 3

Brown 1982; Pettersson et al. 2004; but see Geodakyan et al. 1967), guppies often show female-biased tertiary sex ratios in nature (Haskins et al. 1961; Seghers 1973; Liley and Seghers 1975; Rodd and Reznick 1997; Pettersson et al. 2004). This pattern was replicated in our study, with significantly female-biased tertiary sex ratios in about half of our samples, but with male-biased sex ratios in none of them (Fig. 1). Potential environmental contributors to such biases in nature include sex-specific differences in age-at-maturity (Lovich and Gibbons 1990; Girondot and Pieau 1993), migration patterns (Reynolds et al. 1986; Colwell and Oring 1988) or mortality (Britton and Moser 1982; Hairston et al. 1983). In the present study, we found that sex ratio variation was associated strongly with potential intraguild predators (*Macrobrachium* prawns and perhaps *Rivulus* killifish) and also with a strong determinant of resource levels (canopy cover that influences primary productivity and therefore algal biomass).

Predators can influence tertiary sex ratio by preferentially preying on the sex that is more visible (Hairston et al. 1983), more profitable (Britton and Moser 1982), or easier to catch (Quinn and Kinnison 1999). In guppies, the established hypothesis for female-biased sex ratios is that size-biased predation by *Rivulus* leads to male-biased mortality (Seghers 1973; Liley and Seghers 1975; Magurran 2005). This effect is suggested to arise because *Rivulus* is a size-limited predator and male guppies, which are smaller, should thus be easier to catch than female guppies, which are larger. We find only qualified support for this hypothesis: in only some of our analyses was sex ratio related to proxies for *Rivulus* predation pressure (Fig. 2b; Table 2). One potential reason for this ambiguous result is that our *Rivulus* data might not provide a reliable surrogate for predation pressure. This is certainly possible given the cryptic nature of this species (see Methods). Alternatively, *Rivulus* may not be as important in driving sex ratio variation as has been suggested. Perhaps studies of *Rivulus* predation in the laboratory (Haskins et al. 1961; Seghers 1973; Liley and Seghers 1975; Mattingly and Butler 1994) overestimate their potential influence in the wild. Diet analyses have found little evidence that this species eats substantial numbers of guppies in nature (Seghers 1973; Fraser and Gilliam 1992), although small fish such as guppies may be digested at a relatively high rate (Salvanes et al. 1995). To us, the importance of *Rivulus* to sex ratio variation in nature remains an open question.

In contrast to the ambiguous results for *Rivulus*, we find a very strong tendency for increasingly female-biased sex ratios with increasing *Macrobrachium* CPUE (Figs. 2a, 4; Table 2). Although the importance of *Macrobrachium* as a guppy predator is somewhat controversial (Magurran 2005), they certainly do feed on guppies in the lab (A.E.M. and A.P.H., unpublished data). Moreover, guppy scales have been found in the stomachs of wild *Macrobrachium* (Magurran 2005), and *Macrobrachium* abundance is correlated with guppy behaviour (Magurran and Seghers 1990) and male colour (Endler 1978, 1983; Millar et al. 2006), although perhaps not life history (Rodd and Reznick 1991). If female-biased sex ratios are indeed the result of sex-biased predation, *Macrobrachium* may be as likely a candidate as is *Rivulus*, at least on the north slope of the Northern Range Mountains of Trinidad, where our study was conducted. One way to disentangle the effects of each predator might be to conduct a similar study on the south slope, where *Macrobrachium* is rare but *Rivulus* is common (Magurran 2005; N. Millar, personal communication).

Rivulus and *Macrobrachium* are potential intraguild predators (Polis et al. 1989) because, in addition to preying directly on guppies, they may compete with guppies for resources. *Rivulus* and guppies, at least, are known to compete for some of the same foods (Gilliam et al. 1993), but less is known regarding the diet of *Macrobrachium* in Trinidad. Some overlap with guppies does seem likely, however, given that studies of this prawn family (Palaemonidae) at other sites have found they eat a variety of foods, including smaller prawns, small fish, mollusks, insects, macrophytes, decomposing leaf litter, and algae (Covich and McDowell 1996). Perhaps increasing densities of *Rivulus* and/or *Macrobrachium* reduce food availability for guppies, which may then disproportionately affect males owing to their preoccupation with mating rather than foraging (Dussault and Kramer 1981; Magurran and Seghers 1994; see also the related arguments below). We are not aware of any studies that have examined the role of interspecific competition in driving tertiary sex ratio variation in species where sex change is rare or absent.

The other major contributor to tertiary sex ratio variation in our study populations was canopy cover. In particular, sites with lower canopy openness, and therefore lower productivity (Results; Grether et al. 2001), had increasingly female-biased sex ratios (Figs. 3, 4; Table 2). This result supports the hypothesis that resource limitation can cause sex-biased mortality for the sex with a lower tolerance to starvation (usually males), a trend that has been reported in a variety of vertebrate species (Clutton-Brock et al. 1985; Clutton-Brock 1986). More generally, males of many species are often thought to be more vulnerable to a range of physiological stressors, also including temperature extremes (Snelson 1989), hypoxia (Cech et al. 1985), and parasites (Zuk and McKean 1996). Although not examined here, some of these other factors may also influence guppy sex ratio variation. For instance, immunocompetence might be compromised in the brightly coloured males at low-predation sites (Folstad and Karter 1992; Magurran 2005), which are often the very places where strongly female-biased sex ratios are observed (Seghers 1973; Liley and Seghers 1975; Rodd and Reznick 1997).

In the above discussion, we considered the individual influences of *Rivulus/Macrobrachium* and canopy cover on guppy sex ratio. We also found a potential interaction, such that the effect of canopy openness appeared greatest when *Macrobrachium* CPUE was highest (Fig. 4). Although this interaction had strong statistical support, it must interpreted cautiously owing to a non-random distribution of canopy and *Macrobrachium* values (see Results). Interactions of this sort, such as between predation and resource levels, have been demonstrated for various life history traits in some species (Falconer and Latyszewski 1952; Walsh and Reznick 2008), although usually not in guppies (Reznick 1982; Reznick and Bryga 1996; but see Reznick and Bryga 1987). Our study, however, may be the first to demonstrate such effects for tertiary sex ratio. We can see several possible causes that might warrant further consideration. First, male guppies that are already physically weakened and/or spend a greater proportion of time foraging (because of low resource levels caused by a closed canopy) may be differentially susceptible to *Rivulus/Macrobrachium* predation. Supporting this possibility, male guppies from low resource sites spend more time foraging than do those from high-resource sites (Kolluru and Grether 2005), which could increase their predation risk (Lima 1998). Second, competition with *Rivulus/Macrobrachium* may compound resource limitation in sites with low productivity, again affecting male guppies disproportionately. Third, *Rivulus*/*Macrobrachium* may prey on guppies less frequently when canopy openness is high and other food sources are abundant, but they may feed more frequently on guppies under low resource conditions.

Implications

The most general implication of our study is that it shows the value of simultaneously assessing multiple environmental factors that might influence tertiary sex ratio. In guppies, *Rivulus* predation is an obvious initial explanation for female-biased sex ratios at low-predation sites (Seghers 1973; Liley and Seghers 1975). First, *Rivulus* is common at many low-predation sites but is usually rare at high-predation sites owing to exclusion by dangerous predatory fish (Gilliam et al. 1993), and second, *Rivulus* preys more on male than female guppies in the laboratory (Haskins et al. 1961; Seghers 1973). Although this hypothesis is certainly plausible, it ignores variation in other environmental factors, such as *Macrobrachium* abundance and resource levels. We assessed these multiple factors by sampling a large number of populations where covariance could be partly broken down to better reveal the independent effects of each factor. In doing so, we found that multiple causal factors were important, as well as interactions between them. Future studies of sex ratio variation would benefit from adopting a similar multi-factorial approach.

We would like to close by highlighting the potential value of the guppy system for examining relationships between tertiary, secondary, and primary sex ratios. Given a skew in the tertiary sex ratio, parents might be expected to bias their primary sex ratio towards the locally rarer sex (Fisher 1930; Charnov 1982). This does not seem to be the case in guppies (Haskins et al. 1961; Seghers 1973; Brown 1982; Pettersson et al. 2004; but see Geodakyan et al. 1967), nor in many other vertebrates (Cockburn et al. 2002; Donald 2007). Several explanations for this have been advanced, each of which may be informed by guppies. First, tertiary sex ratio may not accurately reflect differential mating opportunities. This may be particularly true in guppies, where few adult females will be sexually receptive at any given time (Magurran and Seghers 1994). Second, temporal variation may mean that populations do not experience consistently biased tertiary sex ratios (Pettersson et al. 2004), which would therefore not favour the evolution of biased sex ratios at birth. In our study, however, guppy sex ratio variation among sites was reasonably consistent between years (Results; see also Seghers 1973), even despite large differences in overall densities (ESM, Table S2). Third, mortality after the period of parental care is not expected to affect the evolution of primary sex ratio (Fisher 1930; Leigh 1970). The reason is that differential mortality after birth is offset by the resulting opposite differential in mating opportunities. Finally, even if modification of primary sex ratios were adaptive, heterogametic sex determination in vertebrates may make sex ratio adjustment too costly (Cockburn et al. 2002). Given the dramatic tertiary sex ratio variation in guppies, this system would seem a useful one for further examination of these possibilities.

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