

Environmental factors influencing adult sex ratio in *Poecilia reticulata*: laboratory experiments

A. E. MCKELLAR* AND A. P. HENDRY

Redpath Museum and Department of Biology, McGill University, 859 Sherbrooke Street West,
Montréal, QC, H3A 2K6 Canada

(Received 25 February 2011, Accepted 19 June 2011)

The potential causes of adult sex ratio variation in guppies *Poecilia reticulata* were tested in laboratory experiments that evaluated the mortality rates of male and female *P. reticulata* exposed to potential predators (Hart's rivulus *Rivulus hartii* and freshwater prawns *Macrobrachium crenulatum*) and to different resource levels. *Poecilia reticulata* mortality increased in the presence of *R. hartii* and *M. crenulatum*, and low resource levels had an effect on mortality only in the presence of *M. crenulatum*. *Rivulus hartii* preyed more often on male than on female *P. reticulata*, and this sex-biased predation was not simply the result of males being smaller than females. In contrast, no sex-biased mortality was attributable to *M. crenulatum* or low resource levels. © 2011 The Authors

Journal of Fish Biology © 2011 The Fisheries Society of the British Isles

Key words: predation; resource limitation; sex-biased mortality; guppies; Trinidad.

INTRODUCTION

Sex ratios have a variety of consequences for the ecology and evolution of natural populations. One set of consequences relates to population growth and dispersal (Waage, 1982; Lawrence, 1987; Hazlett *et al.*, 2005). These effects stem from differences between the sexes in reproductive contributions to population growth and to dispersal. Another set of consequences relates to mating behaviour and the opportunity for sexual selection. The most obvious effect here is that increasing numbers of sexually active males relative to females (operational sex ratio, OSR) often increases male–male competition and favours female choice, thus increasing sexual selection on males and promoting the evolution of sexual dimorphism (Emlen & Oring, 1977; Magellan & Magurran, 2007). These and other consequences point to the importance of understanding the causes of sex ratio variation in nature.

Sex ratio variation in animals could have several causes. One of these is variation in sex ratio at birth, whether as a result of environmental sex determination, genetically based biases or epigenetic effects (Kraak & Pen, 2002; Rutkowska & Badyaev, 2008). In many animals, however, sex ratio at birth almost invariably approximates 1:1 (Clutton-Brock & Iason, 1986; Krackow, 2002). In these cases, variation in the

*Author to whom correspondence should be addressed at present address: Department of Biology, Queen's University, Kingston, ON, K7L 3N6 Canada. Tel.: +1 613 533 6000 ext. 77593; email: ann.mckellar@queensu.ca

adult sex ratio (ASR) is the result of factors that cause sex-biased mortality. These factors could include predation (Berger & Gompper, 1999; Sommer, 2000), resource limitation (Klein, 1968), reproductive costs (Promislow, 2003; Liker & Székely, 2005) or parasitism (Zuk & McKean, 1996).

The present research was motivated by a qualitative observation in nature: when collecting guppies *Poecilia reticulata* Peters 1859 for studies of evolutionary diversification, ASR seemed to be strongly biased toward females and highly variable among sites (McKellar *et al.*, 2009). In fact, ASR seemed to vary more among populations than did all of the other *P. reticulata* traits (Liley & Seghers, 1975; Reznick & Endler, 1982; Reznick *et al.*, 1996; Rodd & Reznick, 1997; Magurran, 2005) on which so much research effort has been expended. Moreover, many of those other traits could well be influenced by variation in ASR through its effects on, for example, life history evolution and sexual selection. Given this apparently dramatic variation and its many important consequences for ecology and evolution, it seemed important to understand the causes of this variation. This work began with a correlative field study of ASR variation among natural populations of *P. reticulata* (McKellar *et al.*, 2009). The current study presents the results of laboratory experiments designed to experimentally manipulate the factors that correlated with ASR variation in the field survey.

Adult sex ratio variation in *P. reticulata* appears to have considerable ecological and evolutionary consequences. For instance, differences in OSR, which will be influenced by ASR (Emlen & Oring, 1977), alter *P. reticulata* mating behaviour and the relative importance of mate choice *v.* sexual coercion (Evans & Magurran, 1999; Jirotkul, 1999; Magellan & Magurran, 2007), as well as maternal investment in offspring size (Barbosa & Magurran, 2010). In addition, ASR can influence *P. reticulata* dispersal patterns because males might be more likely to move away from male-biased locations (Croft *et al.*, 2003). Finally, ASR should have particularly strong effects on *P. reticulata* population growth rates: female-biased populations should show much faster growth than male-biased populations because females can store and use sperm from a single mating for multiple reproductive cycles. As in many other species, the underlying causes of ASR variation in *P. reticulata* remain ambiguous (Magurran, 2005). Given that *P. reticulata* sex ratios in newborns and juveniles are typically 1:1 (Haskins *et al.*, 1961; Seghers, 1973; Brown, 1982; Pettersson *et al.*, 2004; for an apparent exception see Geodakyan *et al.*, 1967), ASR variation is most probably caused by sex-biased mortality of adults.

Two main hypotheses have been advanced for sex-biased mortality in *P. reticulata*, both motivated by the observation that ASRs tend to be female-biased mainly in 'low-predation' sites. These sites are typically found in the upper reaches of streams and contain few *P. reticulata* predators that collectively have a weak influence on *P. reticulata* demographics, whereas 'high-predation' sites are found further downstream and contain a variety of predatory fishes that collectively have a stronger effect on *P. reticulata* demographics (Reznick *et al.*, 1996; Rodd & Reznick, 1997; Magurran, 2005). The first hypothesis is that the predators at these low-predation sites, such as the killifish Hart's rivulus *Rivulus hartii* (Boulenger 1890) and freshwater prawns of the genus *Macrobrachium*, are better able to capture and kill male than female *P. reticulata*, perhaps because males are often smaller than females (Seghers, 1973; Liley & Seghers, 1975; Magurran, 2005), and *R. hartii*, at least, is a gape-limited predator (Seghers, 1973). In support of a role for *R. hartii* predation,

previous laboratory studies have found that males (Haskins *et al.*, 1961; Seghers, 1973), and smaller fish (Liley & Seghers, 1975; Mattingly & Butler, 1994), are more vulnerable to *R. hartii* predation, and field surveys found increasingly female-biased ASRs at sites with higher *R. hartii* density (McKellar *et al.*, 2009). In support of a possible role for *Macrobrachium* spp. predation, field surveys found increasingly female-biased ASRs at sites with higher *Macrobrachium* spp. density (McKellar *et al.*, 2009). It should be noted, however, that field-based associations between *R. hartii* or *Macrobrachium* spp. density and *P. reticulata* ASR could result from interspecific competition for resources rather than from predation.

Low-predation *P. reticulata* populations are often associated with lower resource availability (Reznick *et al.*, 2001). Thus, a second major hypothesis for sex-biased mortality in *P. reticulata* is resource limitation. Males are generally more active and spend less time foraging than do females (Dussault & Kramer, 1981), and males might therefore have a greater risk of mortality under resource-limited conditions (Schultz, 1977). Indeed, field surveys found increasingly female-biased ASRs at sites with more closed canopies (McKellar *et al.*, 2009), conditions known to reduce incident light and therefore decrease primary productivity and the availability of *P. reticulata* food (Grether *et al.*, 2001). Field surveys also found that the potential effect of closed canopies was greatest at sites with higher *Macrobrachium* spp. density (McKellar *et al.*, 2009). This result suggests a possible interaction between canopy cover and *Macrobrachium* spp., such that (1) male *P. reticulata* become more vulnerable to predation under resource limitation, or (2) *Macrobrachium* spp. act mainly as competitors rather than as predators and thereby exacerbate the negative effects of resource limitation (McKellar *et al.*, 2009).

Laboratory experiments present an opportunity to better inform the above hypotheses. The goals of this study were to (1) examine the roles of *R. hartii* and *Macrobrachium crenulatum* as *P. reticulata* predators, (2) confirm *R. hartii* as a sex-biased predator and partition the effects of *P. reticulata* sexual size dimorphism *v.* other sex differences with respect to *R. hartii* predation and (3) examine the possible importance of *M. crenulatum* predation and resource limitation in relation to sex-biased mortality.

MATERIALS AND METHODS

LABORATORY CONDITIONS AND EXPERIMENTAL FISH

Two types of *P. reticulata* were used in these experiments ('wild-origin' and 'laboratory-origin'), with most experiments using equal replication of the two types. The use of these two types of fish can be seen as complementary. First, the use of offspring from wild-origin *P. reticulata* minimized the potential effects of adaptation to laboratory conditions, whereas the use of offspring from laboratory-origin *P. reticulata* minimized potential carryover effects from the wild. Second, the use of two types increased the ability to consider whether the results might be general. The two types of *P. reticulata* were always kept separate and no individuals were re-used in any of the experiments or treatments.

Wild-origin *P. reticulata* were the first-generation offspring of fish collected from the Marianne River on the north slope of Trinidad's Northern Range Mountains. The specific site, designated M10 in previous work (10° 46' 21.8" N; 61° 17' 32.6" W; Crispo *et al.*, 2006; Millar *et al.*, 2006; McKellar *et al.*, 2009; Weese *et al.*, 2010), is considered low predation because it contained only 'weak' *P. reticulata* predators (*R. hartii* and *Macrobrachium* spp.)

as opposed to 'strong' predators found in high-predation sites [e.g. *Crenicichla* spp., *Hoplias malabaricus* (Bloch 1794), *Eleotris pisonis* (Gmelin 1789) and *Dormitator maculatus* (Bloch 1792)]. This particular population was chosen because (1) it showed consistent and strongly female-biased ASRs (mean proportion males \pm 95% c.i. = 0.24 ± 0.17 in 2006 and 0.27 ± 0.07 in 2007; McKellar *et al.*, 2009), (2) it contained both *R. hartii* and freshwater prawns of the genus *Macrobrachium* (Millar *et al.*, 2006; McKellar *et al.*, 2009) and (3) it had a closed canopy (mean canopy openness \pm 95% c.i. = $11.7 \pm 3.0\%$) and low resource levels (mean chlorophyll *a* concentration on rocks \pm 95% c.i. = 7.4 ± 3.7 mg chl m⁻²; A. McKellar, unpubl. data). In March (dry season) and November (wet season) of 2007, 30–40 mature females and 20–30 mature males were collected from this site. These fish were transported to a laboratory at McGill University, Canada, where six to eight females and five to seven males were kept per 40.9 cm \times 20.6 cm \times 25.7 cm aquarium equipped with corner filters. Females were inspected daily and those about to give birth were separated into individual 30.8 cm \times 13.7 cm \times 21 cm aquaria. Newborn offspring were removed from their mother's aquarium (*P. reticulata* do not provide parental care) and placed in 40.9 cm \times 20.6 cm \times 25.7 cm aquaria, with 10–15 individuals per aquarium.

The laboratory-origin *P. reticulata* were a mixture of fish descended from a variety of Trinidadian populations collected over the past 5 years. This mixed population was supplemented at various times by some domesticated pet-store 'feeder' *P. reticulata*. The laboratory-origin *P. reticulata* were maintained at self-regulating densities in 91.8 cm \times 32.1 cm \times 38.7 cm aquaria with corner filters, gravel and plastic plants. Mature females were allowed to give birth in the aquaria (*i.e.* they were not isolated), and the young also grew up in the same aquaria.

All adult *P. reticulata* of both types were fed crushed flake-food daily, whereas juveniles were fed live *Artemia* sp. nauplii twice daily. As soon as individuals could be putatively assigned to a sex by the presence of a gonopodium in males and a 'gravid spot' in females, they were used in the experiments described below. Subsequent tracking of fish showed that these putative sex assignments were never incorrect. Using fish at this early stage of maturation helped to reduce the size differences between males and females that increasingly accrue with age (Reznick & Miles, 1989). All fish rearing and experiments were conducted under natural light (45° N) in a greenhouse. This lighting was preferable to abrupt on:off transitions between light and dark because crepuscular activities are probably very important to both *P. reticulata* and predators (Magurran, 2005). The tops of tanks were covered with black cloth, and although tanks were examined daily, no algae could be seen on the tank walls.

Potential predators used in the experiments were *R. hartii* and *M. crenulatum* collected from several streams containing *P. reticulata* in the Marianne and Paria watersheds of Trinidad and transported back to a laboratory at McGill University. *Rivulus hartii* were kept in 61.4 cm \times 31.9 cm \times 41.3 cm aquaria with approximately five individuals per aquarium. *Macrobrachium crenulatum* were kept in 91.8 cm \times 32.1 cm \times 38.7 cm aquaria with approximately three individuals per aquarium. All *R. hartii* and *M. crenulatum* aquaria were equipped with corner filters, gravel and several pieces of clay pots for use as refuges. *Rivulus hartii* were fed once per day, alternating between flake-food and frozen blood worms. Their diet was also occasionally supplemented with male or female 'feeder' *P. reticulata* of various sizes. *Macrobrachium crenulatum* were fed three times per week with shrimp pellets and always had 'feeder' *P. reticulata* available in their aquaria. All potential predators thus had experience preying on *P. reticulata* in laboratory aquaria prior to their use in the experiments. Only 'large' *R. hartii* and *M. crenulatum* individuals were used in the experiments (<50 mm total length, L_T ; McKellar *et al.*, 2009), so as to increase the chances of predation.

Direct behavioural observations of *R. hartii* or *M. crenulatum* were not performed during the experiments. Nevertheless, several lines of evidence suggest that they caused most, if not all, *P. reticulata* mortality in the experiments. First, mortality was much lower in no-predator control treatments than in predator treatments. Second, *R. hartii* have repeatedly been observed preying on *P. reticulata* in the laboratory and in the field (Haskins *et al.*, 1961; Seghers, 1973, 1978; Liley & Seghers, 1975; Mattingly & Butler, 1994). Third, *M. crenulatum* have been observed catching *P. reticulata* in the wild and in the laboratory (E. Crispo, A. Hendry, A. McKellar, N. Millar & M. Piette, pers. obs.).

PREDATION-RESOURCE EXPERIMENTS

Two types of predation and resource experiments were performed: *R. hartii* experiments and *M. crenulatum* experiments (Appendix). All experiments began with 10 male and 10 female *P. reticulata* in a 50.4 cm × 26.8 cm × 30.6 cm aquarium. *Rivulus hartii* experiments included either a single *R. hartii* (predator treatment) or no predator (control treatment), gravel, a piece of clay pot, a corner filter and water to a depth of 21 cm. *Macrobrachium crenulatum* experiments included either two *M. crenulatum* (predator treatment) or none (control treatment), two pieces of clay pot, a piece of plastic plant and water to a depth of 11 cm. The water was shallower in the *M. crenulatum* experiments so as to increase potential capture rates by this benthic-oriented species. Although 11 cm is not the typical depth in natural stream populations of *P. reticulata*, this shallower depth was used in the *M. crenulatum* experiments so as to increase potential capture rates by this benthic-oriented species. In addition, both *P. reticulata* and *Macrobrachium* spp. are sometimes found at similar depths (Croft *et al.*, 2004; Millar *et al.*, 2006), and they have been used in some previous experiments (Gilliam *et al.*, 1993; Chapman *et al.*, 2010).

Each experiment consisted of two additional treatments: high resources and low resources. *Poecilia reticulata* in high-resource treatments were fed live *Artemia* sp. nauplii *ad libitum*. *Poecilia reticulata* in low-resource treatments were starved for 7 days preceding and throughout the treatment. This particular method for imposing resource limitation was chosen because pilot studies revealed that *P. reticulata* under these conditions gradually decreased in relative mass (mass change over 14 days ± 95% c.i. = 18.9 ± 27.2%). This decrease suggests nutritional stress that might mimic the effects of resource limitation, although resource limitation in nature will probably work differently. *Artemia* sp. nauplii were chosen as food for *P. reticulata* in the treatments because there is no evidence that *M. crenulatum* or adult *R. hartii* will eat *Artemia* sp. nauplii in the laboratory (Fraser *et al.*, 1999; A. McKellar, pers. obs.), although juvenile *R. hartii* certainly do (Walsh & Reznick, 2008). The hope was that potential predators would thereby have similar food levels in both low- and high-resource treatments.

One complete experiment involved 10 male and 10 female *P. reticulata* that were either wild-origin or laboratory-origin in each combination of resource treatments (high or low) crossed with predator treatments (present or absent) (Appendix). The two resource treatments were run one after the other (with different *P. reticulata*) so as to use the same individual predator (*R. hartii*) or predator pairs (*M. crenulatum*) in both resource treatments for a given experiment. Replication was achieved by running multiple predator experiments of each type (*R. hartii* or *M. crenulatum*) with new sets of *P. reticulata*, with the order of the resource treatments reversed between the different experiments. For example, *R. hartii* individual one might be used in a low-resource treatment with 10 male and 10 female *P. reticulata* and subsequently in a high-resource treatment with 10 new males and 10 new females. *Rivulus hartii* individual two would then be used in a new experiment with new *P. reticulata* in the same treatments but with the low- and high-resource treatments in the opposite order. A total of six *R. hartii* experiments (four predator treatments and two no-predator controls) and five *M. crenulatum* experiments (four predator treatments and one no-predator control) were completed (Appendix). Given the large number of *P. reticulata* needed for the experiments, the number of predator treatments in relation to control treatments was maximized. Nonetheless, it is believed that the number of control treatments was sufficient for the purposes of this study because (1) no *P. reticulata* mortality was observed in *R. hartii* control treatments, and (2) despite *P. reticulata* mortality in the *M. crenulatum* control experiment, mortality was still greater when *M. crenulatum* were present.

Prior to the start of each experiment, *P. reticulata* were anaesthetized with MS-222 and their standard lengths (L_S) recorded with a ruler. Each group of 10 males and 10 females was placed in an experimental aquarium and allowed 24 h to acclimate. Potential predators were then added to the predator treatments. The number of male and female *P. reticulata* remaining in the aquarium was counted every 24 h. Experiments ended when either (1) all *P. reticulata* in the aquarium were gone, (2) 30 days passed or (3) the predators went for 7 days without consuming any *P. reticulata*.

SIZE-BIASED PREDATION EXPERIMENTS

The above experiments showed that *R. hartii* preyed more often on male than female *P. reticulata*, and it was of interest whether this result was simply due to males being smaller, or whether other differences between the sexes could also be important. Although sexual size dimorphism was reduced in the above experiments by using newly matured individuals, females were still larger on average than were males (e.g. *R. hartii* experiments: $L_S \pm 95\%$ c.i. = 16.0 ± 0.3 mm for males and 17.2 ± 0.4 mm for females; *t*-test, $t = 4.55$, $n = 160$, $P < 0.001$). Therefore, additional *R. hartii* experiments were performed to disentangle the effects of *P. reticulata* size from other effects of sex. This was done by individually marking the fish and then including L_S as a covariate in the statistical analyses. Similar results were found when substituting mass or body depth for L_S , and so these other analyses are not shown.

Prior to the start of each size-biased predation experiment, *P. reticulata* were anaesthetized with MS-222. Each individual was then tagged with a unique combination of visible implant elastomers (Northwest Marine Technology; <http://nmt.US/>), a method proven effective in previous work (Reznick *et al.*, 1996; Croft *et al.*, 2003; van Oosterhout *et al.*, 2007; Weese *et al.*, 2010). The same colour was used on all individuals in a given experiment (to avoid the possibility of colour-biased predation), but the location of tag placement was varied, with two tags per fish. Tag locations were on the left or right side of the fish in (1) the tail muscle below or above the lateral line and (2) two places next to the dorsal fin. Equivalent tag locations were used on males and females in a given experiment (as sex could easily be identified), and fish were chosen randomly with respect to size for tag placement. Standard length was then recorded with a ruler. Each experiment consisted of 10 males and 10 females, all of which were reared under high resource levels. A total of four experiments were completed, two with wild-origin and two with laboratory-origin *P. reticulata*, each with a unique *R. hartii* (Appendix). In addition, a control experiment was performed ($n = 20$ males, $n = 20$ females) to test for the effects of tagging on *P. reticulata* survival. Only one fish died over a 30 day period in this control experiment, and so tagging-induced mortality should not influence the results. All other conditions and procedures were the same as in the above predation–resource experiments. All methods used in this study comply with current laws of the country in which they were performed and with an animal use protocol (#4570) from McGill University. Exposing live animals to their natural predators was necessary in order to determine the environmental conditions that may be responsible for biased sex ratios in natural populations. Survival analysis typically requires considerable sample sizes, but an effort was made to keep the number of fish used as live prey to the minimum necessary to obtain these unique results.

DATA ANALYSIS

All statistical analyses were performed in R v. 2.11.1 (R Foundation for Statistical Computing 2010; www.r-project.org). Survival analysis was used to model the time to death of *P. reticulata* in each experiment. Survival analysis has the advantage of including ‘censored’ information on individuals that are still alive at the end of the experiment (Lee & Wang, 2003). The Cox proportional hazards method was used to model the probability of *P. reticulata* death in a particular time interval, given survival up to that time interval (‘hazard function’), as a function of various predictor variables (Lee & Wang, 2003). The Cox method is semi-parametric and does not require the choice of a specific probability distribution to represent survival times (Cox, 1972). Regardless, similar conclusions were obtained when using parametric model distributions (Weibull, exponential and lognormal).

The effects of various predictor variables on survival were analysed separately for each type of experiment: *R. hartii* predation–resource, *M. crenulatum* predation–resource, and *R. hartii* size-biased predation. This approach was used to infer causes of mortality in the presence of each type of predator, rather than to directly compare the effects of different types of potential predators. For the predation–resource experiments, predictor variables were *P. reticulata* sex, resource level, presence or absence of predator (for *M. crenulatum* experiments only), and all two-way interactions. The sex-by-resource level interaction term was of particular interest because field surveys suggested that the combination of *Macrobrachium* spp. and high canopy cover (reflecting low resource levels) was particularly predictive of male-biased sex

ratios (McKellar *et al.*, 2009). For the size-biased predation experiments, predictor variables included *P. reticulata* sex and L_S and their interaction. Minimum adequate models were then identified by stepwise deletion of non-significant terms.

In addition, each of the three survival models (*R. hartii* predation–resource, *M. crenulatum* predation–resource, and *R. hartii* size-biased predation) included a shared frailty term corresponding to the experiment number. Shared frailty in survival models is essentially analogous to random effects in linear regression and thus accounted for correlations between groups of observations (Petersen, 1998). As each individual experiment within the three experiment types involved a unique individual predator (or predator pair) and either laboratory or wild *P. reticulata*, the inclusion of this frailty term took into account potential differences owing to these factors (Smith & Blumstein, 2010). Thus, different experiments within each model were allowed to have different baseline mortality rates. Frailty was modelled with a gamma distribution, but similar conclusions were obtained using a Gaussian distribution.

RESULTS

All *P. reticulata* in the no-predator control groups in the *R. hartii* predation–resource experiments survived the entire 30 day period. This result indicates that mortality experienced when *R. hartii* were present was probably due to the predators themselves. It also indicates that different resource levels alone, at least as imposed and tested in this study, had no effect on the mortality of *P. reticulata* in these experiments. Thus, control experiments were not included in survival models for *R. hartii* predation–resource experiments (*i.e.* no ‘presence or absence of predator’ term). Survival rates for each *R. hartii* predation–resource experiment where predators were present are shown in Fig. 1(a). Only *P. reticulata* sex remained in the final model (Table I), with males having significantly lower survival than females [Fig. 1(b)]. From this it is inferred that *R. hartii* were responsible for *P. reticulata* mortality and that males were more vulnerable to *R. hartii* predation than were females.

Survival rates for each *M. crenulatum* predation–resource experiment are shown in Fig. 2(a). Both resource level and presence or absence of predator remained in the final model (Table I), with *P. reticulata* on low-resource diets having significantly lower survival than those on high-resource diets [Fig. 2(b)], and *P. reticulata* exposed to *M. crenulatum* having significantly lower survival than control fish [Fig. 2(c)]. From this it is inferred that *M. crenulatum* and low resource levels contributed to *P. reticulata* mortality in *M. crenulatum* experiments, but that *P. reticulata* sex had no significant effect on their survival.

Survival rates for each *R. hartii* size-biased predation experiment are shown in Fig. 3(a). Only *P. reticulata* sex remained in the final model (Table I), with males again having significantly lower survival than females [Fig. 3(b)]. From this it is inferred that, even after taking length into account, males were still more vulnerable to *R. hartii* predation than were females.

DISCUSSION

Although the consequences of sex ratio variation have been studied in detail (Emlen & Oring, 1977; Lawrence, 1987; Hazlett *et al.*, 2005; Magellan & Magurran, 2007), the purpose of this study was to explore some of its causes. Two potential causes of sex-biased mortality, and therefore ASR variation, are predation and

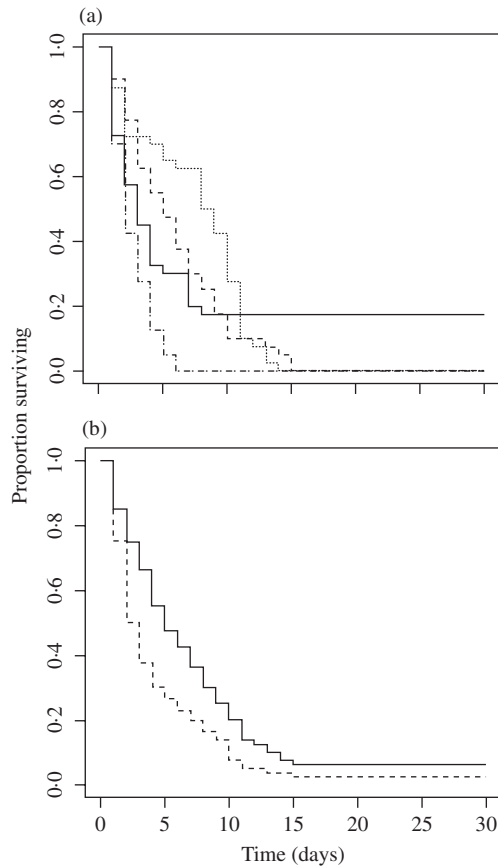


FIG. 1. Proportion of *Poecilia reticulata* surviving up to 30 days in *Rivulus hartii* predation–resource experiments showing (a) variation among all four experiments where predators were present (sexes and resource levels pooled) and (b) lower survival for males (---) than for females (—) (experiments pooled).

resource limitation (Klein, 1968; Berger & Gompper, 1999; Sommer, 2000). In *P. reticulata*, field surveys found that both of these factors were correlated with ASR variation in nature (McKellar *et al.*, 2009). In an attempt to gain some insight into causality in these associations, controlled laboratory experiments were implemented that exposed groups of *P. reticulata* to different potential predators and resource levels. Based on the results of these experiments, the factors driving *P. reticulata* mortality independent of any sex bias, the potential contribution of these factors to sex-biased mortality, and a discussion of implications for the study of ASR variation in nature are outlined below.

THE DRIVERS OF *P. RETICULATA* MORTALITY

Several lines of evidence suggest that *R. hartii*, *Macrobrachium* spp., and resource levels (the last often based on canopy cover as a surrogate) all contribute to the mortality of *P. reticulata*. Evidence from nature includes associations between some or all of the above factors and *P. reticulata* life history (Reznick *et al.*, 1996, 2001;

TABLE I. Cox proportional hazard models testing for the effects of predictor variables on *Poecilia reticulata* survival in three types of experiments. Each model included 'experiment number' as a shared frailty term to allow for different baseline mortality rates among replicate experiments. χ^2 and *P*-values of terms not retained in the final model are those just before removal

Predictor variable	Estimate \pm s.e.	χ^2_1	<i>P</i>
<i>Rivulus hartii</i> predation–resource experiments			
Sex	0.53 \pm 0.16	10.2	0.001
Resource level	–0.30 \pm 0.17	3.32	0.069
Sex \times resource level	–0.01 \pm 0.33	0.02	0.885
<i>Macrobrachium crenulatum</i> predation–resource experiments			
Sex	–0.09 \pm 0.18	0.37	0.541
Resource level	0.10 \pm 0.20	25.36	<0.001
Presence or absence of predator	1.13 \pm 0.46	5.91	0.015
Sex \times resource level	–0.40 \pm 0.37	2.70	0.099
Sex \times presence or absence of predator	–0.24 \pm 0.47	0.28	0.594
Resource level \times presence or absence of predator	0.14 \pm 0.51	0.08	0.772
<i>R. hartii</i> size-biased predation experiments			
Sex	0.61 \pm 0.24	6.18	0.013
L_S	–0.10 \pm 0.08	0.93	0.336
Sex $\times L_S$	–0.10 \pm 0.16	0.83	0.363

L_S , standard length.

Rodd & Reznick, 1997; Grether *et al.*, 2001), male colour (Millar *et al.*, 2006; Schwartz & Hendry, 2010), behaviour (Farr, 1975; Magurran & Seghers, 1990) and ASR (McKellar *et al.*, 2009). In addition, the stomachs of wild *R. hartii* and *Macrobrachium* spp. sometimes contain *P. reticulata* (Seghers, 1973; Magurran, 2005). Evidence from laboratory studies includes experiments showing that *R. hartii* feed readily on *P. reticulata* under a variety of conditions (Haskins *et al.*, 1961; Seghers, 1973; Liley & Seghers, 1975; Mattingly & Butler, 1994), and this is the first study confirming that *P. reticulata* mortality increases in the presence of *M. crenulatum* [Table I and Fig. 2(c)]. One important caveat here is that mortality rates were reasonably high in these last experiments even when *M. crenulatum* were absent [Fig. 2(c)]. Given the lower water levels in *M. crenulatum* experiments in comparison to *R. hartii* experiments, water quality and undetected disease could have contributed to this increased *P. reticulata* mortality. In any case, *P. reticulata* exposed to *M. crenulatum* still died at higher rates than those not exposed to *M. crenulatum*. *Macrobrachium crenulatum* thus increased mortality rates over and above whatever other factor was causing mortality in these experiments.

In addition, the present laboratory experiments showed that a manipulation of resource levels influenced *P. reticulata* mortality, although only in the presence of some predators. Specifically, *P. reticulata* in *M. crenulatum* experiments died at significantly higher rates under low resource levels than under high resource levels [Table I and Fig. 2(b)]. This result might have at least two causes. On the one hand, individuals in low-resource environments might be more susceptible to predation because they are weaker, spend more time searching for food or attempt to forage in

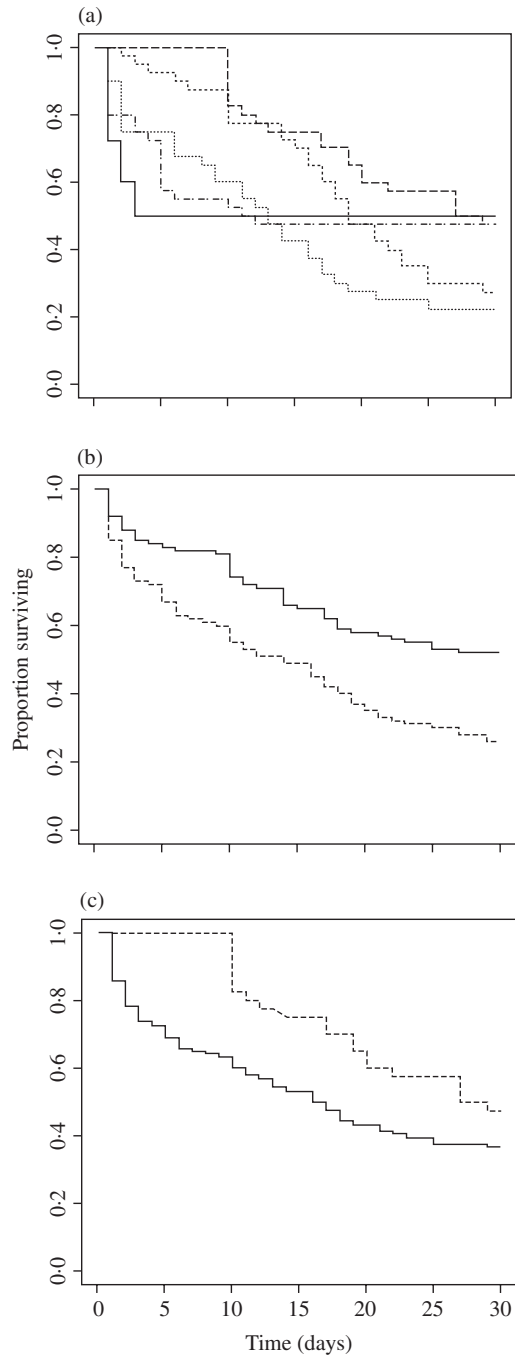


FIG. 2. Proportion of *Poecilia reticulata* surviving up to 30 days in *Macrobrachium crenulatum* predation–resource experiments showing (a) variation among all five experiments (sexes and resource levels pooled), (b) higher survival for fish on high-resource diets (—) than for fish on low-resource diets (---) (experiments pooled), and (c) lower survival for fish exposed to *M. crenulatum* (—) than for control fish (---) (experiments pooled).

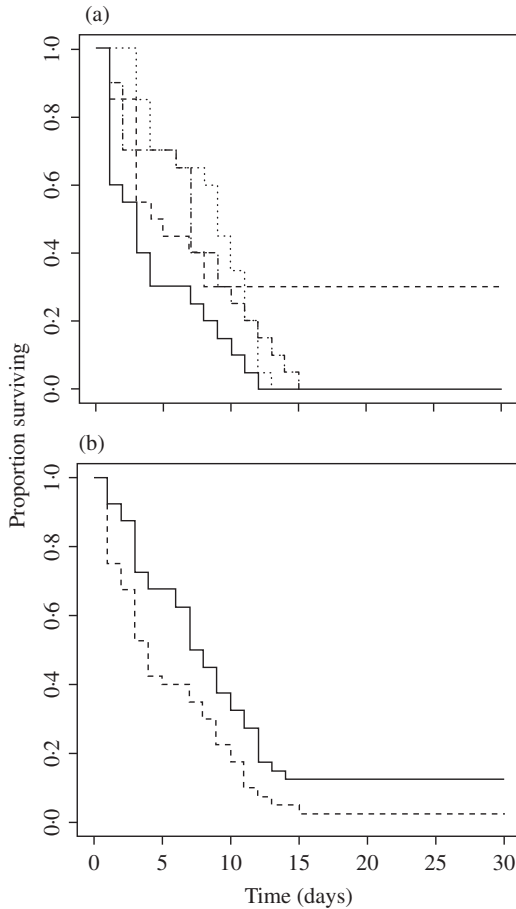


FIG. 3. Proportion of *Poecilia reticulata* surviving up to 30 days in *Rivulus hartii* size-biased predation experiments showing (a) variation among all four trials where predators were present (sexes pooled) and (b) lower survival for males (—) than for females (---) (experiments pooled).

more risky locations (Lima, 1998). On the other hand, *M. crenulatum* might compete with *P. reticulata* for the few resources (e.g. small amounts of periphyton) that could have been present in the tanks to which food was not added.

SEX-BIASED MORTALITY

Previous laboratory studies have found that more male *P. reticulata* die than do females when both are held in the presence of *R. hartii* (Haskins *et al.*, 1961; Seghers, 1973), and the present findings were similar [Table I and Figs 1(b) and 3(b)]. Possible causes of this sex-biased mortality include the greater visibility of colourful males relative to drab females (Haskins *et al.*, 1961), the smaller size of males relative to females (Seghers, 1973; Liley & Seghers, 1975; Mattingly & Butler, 1994) and the greater vigilance of females relative to males (Magurran & Nowak, 1991). The second hypothesis, sexual size dimorphism, has been explored in experiments

that controlled for size differences, but results have been variable in that size has sometimes, but not always, been shown to influence predation risk regardless of sex (Seghers, 1973; Liley & Seghers, 1975). To provide a clearer test for such effects, individually marked fish of both sexes were used in order to disentangle the effects of size differences from other potential sex differences. These experiments revealed that sex-biased mortality was not the result of size differences, at least among the sizes that were tested (Table I). This result parallels the finding that, in the wild, male *P. reticulata* have lower recapture probabilities at *R. hartii* sites than do equal-sized females (Reznick *et al.*, 1996).

In contrast to the above confirmation that sex-biased mortality can be caused by *R. hartii*, the present laboratory experiments did not detect sex-biased mortality associated with *M. crenulatum*, low resource levels or their interaction (Table I). These results appear to conflict with field surveys that documented associations between ASR and *Macrobrachium* spp. density, canopy cover and their interaction (McKellar *et al.*, 2009). Possible explanations for differences between the results of laboratory experiments and field studies fall into two general categories: unrealistic laboratory conditions or additional causal factors in nature.

Laboratory aquaria certainly do not replicate nature. For example, *P. reticulata* and their predators are much more confined in laboratory aquaria than they are in nature, whereas many more predators are present in natural pools (Millar *et al.*, 2006; McKellar *et al.*, 2009). Other un-natural conditions in the laboratory include lighting, water depths and flow rates. The potential influence of some of these variables could be explored by using larger aquaria with more complex habitats, or by using semi-natural ponds. In addition, the resource level treatments used in this study were quite different (brief periods of food deprivation) than they would be in nature (prolonged food shortages), which might be addressed by rearing fish for longer periods on low- and high-resource diets. The difficulty here, and the reason this approach was not used, is that different diets over longer time periods will alter growth rates (Reznick, 1983) and thus influence adult body size.

Additional environmental factors that could confound or spuriously generate correlations between ASR and predator densities or resource levels in nature include environment-dependent sex-specific differences in reproductive costs (Promislow, 2003; Liker & Székely, 2005), maturity schedules (Lovich & Gibbons, 1990), dispersal patterns (Reynolds *et al.*, 1986) and parasitism (Zuk & McKean, 1996). All of these factors are potentially important in natural *P. reticulata* populations because they could generate either sex-biased mortality or apparent ASR skews. In terms of reproductive costs, *P. reticulata* in different environments differ dramatically in female reproductive effort (Reznick & Endler, 1982) and male colour (Millar *et al.*, 2006), and this variation can influence energetics and immunocompetence (Reznick, 1983; Grether *et al.*, 2004; Magurran, 2005), thus potentially affecting survival rates. *Poecilia reticulata* populations can also differ greatly in both male and female age-at-maturity (Reznick & Endler, 1982). Males are more likely than females to move between pools (Croft *et al.*, 2003), and the strength of this pattern may vary spatially based on differences in swimming performance (Nicoletto & Kodric-Brown, 1999). Finally, rates of parasite infection vary among sites, and the recapture rates of males, but not females, decline with parasite load (van Oosterhout *et al.*, 2007). In short, many opportunities exist for investigating interactions between multiple causal factors that might influence ASR variation in this species.

IMPLICATIONS

The weight of evidence from a number of studies points to the importance of *R. hartii* predation in generating female-biased ASRs in low-predation *P. reticulata* populations. The present results amplify the strength and generality of this effect, and additionally show that it is probably driven by differences between the sexes that cannot be attributed to just body size. At the same time, this research identifies multiple interacting factors as potentially important to ASR variation in nature. Recent work has suggested similar complexities in other animals. As one example, ASR variation in red deer *Cervus elaphus* depends on juvenile mortality, adult mortality and migration rates, all of which are influenced by density-dependent processes (Clutton-Brock *et al.*, 2002). As another example, male-biased ASRs in wild bird populations are probably the result of sex-specific dispersal, metabolic demands and predation (Donald, 2007).

The complexity inherent in multiple causality suggests the need for a new approach to the study of ASR variation. Of particular use will be controlled experiments involving multiple factors, as well as interactions among them. Such experiments can break typical covariances among factors and help to pinpoint the precise causes of ASR variation. Furthermore, such experiments could be performed in more natural environments, rather than just in the laboratory. Experiments in nature have not yet been applied to studies of ASR variation, but doing so would simultaneously increase realism and allow experimental manipulation of potential causal factors. Although experiments of this sort would not be possible for many organisms, they certainly would be for *P. reticulata*.

M. Boisjoly, J.-S. Moore and A. Schwartz helped collect fish in the field. M. Boisjoly, O. Dutczak, C. LeBlond, C. Saliba and A. Schwartz helped in the laboratory. E. Crispo and A. Schwartz provided useful comments on an earlier version of the manuscript. The Natural Sciences and Engineering Council of Canada provided financial support in the form of a Discovery grant to A. P. H and a Canada Graduate Scholarship to A. E. M.

References

- Barbosa, M. & Magurran, A. E. (2010). Guppies control offspring size at birth in response to differences in population sex ratio. *Biological Journal of the Linnean Society* **100**, 414–419. doi: 10.1111/j.1095-8312.2010.01425.x
- Berger, J. & Gompper, M. E. (1999). Sex ratios in extant ungulates: products of contemporary predation or past life histories? *Journal of Mammalogy* **80**, 1084–1113.
- Brown, L. P. (1982). Can guppies adjust the sex ratio? *American Naturalist* **120**, 694–698.
- Chapman, B. B., Morrell, L. J. & Krause, J. (2010). Unpredictability in food supply during early life influences boldness in fish. *Behavioral Ecology* **21**, 501–506. doi: 10.1093/beheco/arq003
- Clutton-Brock, T. H. & Iason, G. R. (1986). Sex-ratio variation in mammals. *Quarterly Review of Biology* **61**, 339–374.
- Clutton-Brock, T. H., Coulson, T. N., Milner-Gulland, E. J., Thomson, D. & Armstrong, H. M. (2002). Sex differences in emigration and mortality affect optimal management of deer populations. *Nature* **415**, 633–637. doi: 10.1038/415633a
- Cox, D. (1972). Regression models and life tables. *Journal of the Royal Statistical Society B* **34**, 187–220.
- Crispo, E., Bentzen, P., Reznick, D. N., Kinnison, M. T. & Hendry, A. P. (2006). The relative influence of natural selection and geography on gene flow in guppies. *Molecular Ecology* **15**, 49–62. doi: 10.1111/j.1365-294X.2005.02764.x

- Croft, D. P., Albanese, B., Arrowsmith, B. J., Botham, M., Webster, M. & Krause, J. (2003). Sex-biased movement in the guppy (*Poecilia reticulata*). *Oecologia* **137**, 62–68. doi: 10.1007/s00442-003-1268-6
- Croft, D. P., Botham, M. S. & Krause, J. (2004). Is sexual segregation in the guppy, *Poecilia reticulata*, consistent with the predation risk hypothesis? *Environmental Biology of Fishes* **71**, 127–133. doi: 10.1007/s10641-003-0092-5
- Donald, P. F. (2007). Adult sex ratios in wild bird populations. *Ibis* **149**, 671–692. doi: 10.1111/j.1474-919x.2007.00724.x
- Dussault, G. V. & Kramer, D. L. (1981). Food and feeding behaviour of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Canadian Journal of Zoology* **59**, 684–701.
- Emlen, S. T. & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science* **197**, 215–223.
- Evans, J. P. & Magurran, A. E. (1999). Male mating behaviour and sperm production characteristics under varying sperm competition risk in guppies. *Animal Behaviour* **58**, 1001–1006. doi: 10.1006/anbe.1999.1212
- Farr, J. A. (1975). The role of predation in the evolution of social behavior of natural populations of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Evolution* **29**, 151–158.
- Fraser, D. F., Gilliam, J. F., MacGowan, M. P., Arcaro, C. M. & Guillozet, P. H. (1999). Habitat quality in a hostile river corridor. *Ecology* **80**, 597–607. doi: 10.1890/0012-9658(1999)080[0597:HQAHR]2.0.CO;2
- Geodakyan, V. A., Kosobutsky, V. I. & Bileva, D. S. (1967). Regulation of sex ratio by negative feedback. *Genetika* **3**, 152–163.
- Gilliam, J. F., Fraser, D. F. & Alkins-Koo, M. (1993). Structure of a tropical stream fish community: a role for biotic interactions. *Ecology* **74**, 1856–1870.
- Grether, G. F., Millie, D. F., Bryant, M. J., Reznick, D. N. & Mayea, W. (2001). Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology* **82**, 1546–1559. doi: 10.1890/0012-9658(2001)082[1546:RFCCRA]2.0.CO;2
- Grether, G. F., Kasahara, S., Kolluru, G. R. & Cooper, E. L. (2004). Sex-specific effects of carotenoid intake on the immunological response to allografts in guppies (*Poecilia reticulata*). *Proceedings of the Royal Society B* **271**, 45–49. doi: 10.1098/rspb.2003.2526
- Haskins, C. P., Haskins, E. F., McLaughlin, J. J. A. & Hewitt, R. E. (1961). Polymorphism and population structure in *Lebistes reticulatus*, an ecological study. In *Vertebrate Speciation* (Blair, W. F., ed.), pp. 320–395. Austin, TX: University of Texas Press.
- Hazlett, B. A., Bach, C. E., Thompson, G. A. & McLay, C. L. (2005). Movement of male *Heterozius rotundifrons* (Crustacea: Decapoda: Brachyura) depends upon local sex ratio. *New Zealand Journal of Marine and Freshwater Research* **39**, 157–163.
- Jirotkul, M. (1999). Operational sex ratio influences female preference and male-male competition in guppies. *Animal Behaviour* **58**, 287–294. doi: 10.1006/anbe.1999.1149
- Klein, D. R. (1968). The introduction, increase, and crash of reindeer on St. Matthew Island. *Journal of Wildlife Management* **32**, 350–367.
- Kraak, S. B. M. & Pen, I. (2002). Sex-determining mechanisms in vertebrates. In *Sex Ratios: Concepts and Research Methods* (Hardy, I. C. W., ed.), pp. 158–177. Cambridge: Cambridge University Press.
- Krackow, S. (2002). Why parental sex ratio manipulation is rare in higher vertebrates. *Ethology* **108**, 1041–1056. doi: 10.1046/j.1439-0310.2002.00843.x
- Lawrence, W. S. (1987). Effects of sex ratio on milkweed beetle emigration from host plant patches. *Ecology* **68**, 539–546.
- Lee, E. T. & Wang, J. W. (2003). *Statistical Methods for Survival Data Analysis*, 3rd edn. New York, NY: Wiley.
- Liker, A. & Székely, T. (2005). Mortality costs of sexual selection and parental care in natural populations of birds. *Evolution* **59**, 890–897. doi: 10.1554/04-560
- Liley, N. R. & Seghers, B. H. (1975). Factors affecting the morphology and behaviour of guppies in Trinidad. In *Function and Evolution in Behaviour* (Baerends, G. P., Beer, C. & Manning, A., eds), pp. 92–118. Oxford: Clarendon Press.
- Lima, S. L. (1998). Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Advances in the Study of Behavior* **27**, 215–290.

- Lovich, J. E. & Gibbons, J. W. (1990). Age at maturity influences adult sex ratio in the turtle *Malaclemys terrapin*. *Oikos* **59**, 126–134.
- Magellan, K. & Magurran, A. E. (2007). Behavioural profiles: individual consistency in male mating behaviour under varying sex ratios. *Animal Behaviour* **74**, 1545–1550.
- Magurran, A. E. (2005). *Evolutionary Ecology: the Trinidadian Guppy*. New York, NY: Oxford University Press.
- Magurran, A. E. & Nowak, M. A. (1991). Another battle of the sexes: the consequences of sexual asymmetry in mating costs and predation risk in the guppy, *Poecilia reticulata*. *Proceedings of the Royal Society B* **246**, 31–38.
- Magurran, A. E. & Seghers, B. H. (1990). Population differences in predator recognition and attack cone avoidance in the guppy *Poecilia reticulata*. *Animal Behaviour* **40**, 443–452. doi: 10.1016/S0003-3472(05)80524-X
- Mattingly, H. T. & Butler, M. J. (1994). Laboratory predation on the Trinidadian guppy: implications for the size-selective predation hypothesis and guppy life history evolution. *Oikos* **69**, 54–64.
- McKellar, A. E., Turcotte, M. M. & Hendry, A. P. (2009). Environmental factors influencing adult sex ratio in Trinidadian guppies. *Oecologia* **159**, 735–745. doi: 10.1007/s00442-008-1257-x
- Millar, N. P., Reznick, D. N., Kinnison, M. T. & Hendry, A. P. (2006). Disentangling the selective factors that act on male colour in wild guppies. *Oikos* **113**, 1–12. doi: 10.1111/j.0030-1299.2006.14038.x
- Nicoletto, P. F. & Kodric-Brown, A. (1999). The relationship among swimming performance, courtship behavior, and carotenoid pigmentation of guppies in four rivers of Trinidad. *Environmental Biology of Fishes* **55**, 227–235. doi: 10.1023/A:1007587809618
- van Oosterhout, C., Mohammed, R. S., Hansen, H., Archard, G. A., McMullan, M., Weese, D. J. & Cable, J. (2007). Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *International Journal for Parasitology* **37**, 805–812. doi: 10.1016/j.ijpara.2006.12.016
- Petersen, J. H. (1998). An additive frailty model for correlated life times. *Biometrics* **54**, 646–661.
- Pettersson, L. B., Ramnarine, I. W., Becher, S. A., Mahabir, R. & Magurran, A. E. (2004). Sex ratio dynamics and fluctuating selection pressures in natural populations of the Trinidadian guppy, *Poecilia reticulata*. *Behavioral Ecology and Sociobiology* **55**, 461–468. doi: 10.1007/s00265-003-0727-8
- Promislow, D. (2003). Mate choice, sexual conflict, and evolution of senescence. *Behavior Genetics* **33**, 191–201. doi: 10.1023/A:1022562103669
- Reynolds, J. D., Colwell, M. A. & Cooke, F. (1986). Sexual selection and spring arrival times of red-necked and Wilson's phalaropes. *Behavioral Ecology and Sociobiology* **18**, 303–310.
- Reznick, D. N. (1983). The structure of guppy life histories: the trade-off between growth and reproduction. *Ecology* **64**, 862–873.
- Reznick, D. N. & Endler, J. A. (1982). The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**, 160–177.
- Reznick, D. N. & Miles, D. B. (1989). A review of life history patterns in poeciliid fishes. In *Ecology and Evolution of Livebearing Fishes (Poeciliidae)* (Meffe, G. K & Snelson, F. F. Jr, eds), pp. 125–148. Englewood Cliffs, NJ: Prentice Hall.
- Reznick, D. N., Butler, M. J., Rodd, F. H. & Ross, P. (1996). Life-history evolution in guppies (*Poecilia reticulata*). VI. Differential mortality as a mechanism for natural selection. *Evolution* **50**, 1651–1660.
- Reznick, D. N., Butler, M. J. & Rodd, H. (2001). Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *American Naturalist* **157**, 126–140. doi: 10.1086/318627
- Rodd, F. H. & Reznick, D. N. (1997). Variation in the demography of guppy populations: the importance of predation and life histories. *Ecology* **78**, 405–418. doi: 10.1890/0012-9658(1997)078[0405:VITDOG]2.0.CO;2
- Rutkowska, J. & Badyaev, A. V. (2008). Meiotic drive and sex determination: molecular and cytological mechanisms of sex ratio adjustment in birds. *Proceedings of the Royal Society B* **363**, 1675–1686. doi: 10.1098/rstb.2007.0006

- Schultz, R. J. (1977). Evolution and ecology of unisexual fishes. *Evolutionary Biology* **10**, 277–331.
- Schwartz, A. K. & Hendry, A. P. (2010). Testing the influence of local forest canopy clearing on phenotypic variation in Trinidadian guppies. *Functional Ecology* **24**, 354–364. doi: 10.1111/j.1365-2435.2009.01652.x
- Seghers, B. H. (1973). An analysis of geographic variation in the antipredator adaptations of the guppy *Poecilia reticulata*. PhD thesis, Department of Zoology, University of British Columbia, Vancouver, BC, Canada.
- Seghers, B. H. (1978). Feeding behaviour and terrestrial locomotion in the cyprinodontid fish, *Rivulus hartii* (Boulenger). *Verhandlungen, Internationale Vereinigung für theoretische und angewandte Limnologie* **20**, 2055–2059.
- Smith, B. R. & Blumstein, D. R. (2010). Behavioral types as predictors of survival in Trinidadian guppies (*Poecilia reticulata*). *Behavioral Ecology* **21**, 919–926. doi: 10.1093/beheco/arq084
- Sommer, S. (2000). Sex-specific predation on a monogamous rat, *Hypogeomys antimena* (Muridae: Nesomyinae). *Animal Behaviour* **59**, 1087–1094. doi: 10.1006/anbe.2000.1381
- Waage, J. K. (1982). Sex ratio and population dynamics of natural enemies – some possible interactions. *Annals of Applied Biology* **101**, 159–164.
- Walsh, M. R. & Reznick, D. N. (2008). Interactions between the direct and indirect effects of predators determine life history evolution in a killifish. *Proceedings of the National Academy of Sciences of the USA* **105**, 594–599. doi: 10.1073/pnas.0710051105
- Weese, D., Gordon, S. P., Hendry, A. P. & Kinnison, M. T. (2010). Spatiotemporal variation in linear natural selection on body color in wild guppies (*Poecilia reticulata*). *Evolution* **64**, 1802–1815. doi: 10.1111/j.1558-5646.2010.00945.x
- Zuk, M. & McKean, K. A. (1996). Sex differences in parasite infections: patterns and processes. *International Journal for Parasitology* **26**, 1009–1024. doi: 10.1016/S0020-7519(96)00086-0

APPENDIX. For each type of experiment, the presence of predator, number of experiments performed, number of resource treatments per experiment, number of unique predators and total number of *Poecilia reticulata*

Experiment type	Predator treatment	Number of experiments ^a	Number of resource treatments ^b per experiment	Number of unique predators	Total number <i>P. reticulata</i>
<i>Rivulus hartii</i> predation–resource	Predator	2 W, 2 L	1 HR, 1 LR	4	4 experiments × 2 treatments per experiment × 20 fish per treatment = 160
	Control	1 W, 1 L	1 HR, 1 LR	NA	2 experiments × 2 treatments per experiment × 20 fish per treatment = 80
<i>Macrobrachium crenulatum</i> predation–resource	Predator	2 W, 2 L	1 HR, 1 LR	8	4 experiments × 2 treatments per experiment × 20 fish per treatment = 160
	Control	1 L	1 HR, 1 LR	NA	1 experiment × 2 treatments per experiment × 20 fish per treatment = 40
<i>R. hartii</i> size-biased predation	Predator	2 W, 2 L	1 HR	4	4 experiments × 1 treatment per experiment × 20 fish per treatment = 80

^aW, wild-origin *P. reticulata*; L, laboratory-origin *P. reticulata*.

^bHR, high-resource diet; LR, low-resource diet.