

REPLICATED ORIGIN OF FEMALE-BIASED ADULT SEX RATIO IN INTRODUCED POPULATIONS OF THE TRINIDADIAN GUPPY (*POECILIA RETICULATA*)

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There are many theoretical and empirical studies explaining variation in offspring sex ratio but relatively few that explain variation in adult sex ratio. Adult sex ratios are important because biased sex ratios can be a driver of sexual selection and will reduce effective population size, affecting population persistence and shapes how populations respond to natural selection. Previous work on guppies (*Poecilia reticulata*) gives mixed results, usually showing a female-biased adult sex ratio. However, a detailed analysis showed that this bias varied dramatically throughout a year and with no consistent sex bias. We used a mark-recapture approach to examine the origin and consistency of female-biased sex ratio in four replicated introductions. We show that female-biased sex ratio arises predictably and is a consequence of higher male mortality and longer female life spans with little effect of offspring sex ratio. Inconsistencies with previous studies are likely due to sampling methods and sampling design, which should be less of an issue with mark-recapture techniques. Together with other long-term mark-recapture studies, our study suggests that bias in offspring sex ratio rarely contributes to adult sex ratio in vertebrates. Rather, sex differences in adult survival rates and longevity determine vertebrate adult sex ratio.

KEY WORDS: Life span, mark-recapture, population structure, sex-biased mortality, sex-biased recruitment, sex-ratio.

The theory of sex-ratio variation has been heralded as one of the triumphs of evolutionary biology because its predictions have been repeatedly tested and upheld (Charnov 1982; Godfray and Werren 1996; West et al. 2002; Donald 2007). A large body of theoretical papers (e.g., Hamilton 1967; Trivers and Willard 1973; Charnov 1982) predicts when and why offspring sex ratios diverge from the expected ratio of 1:1 (Fisher 1999) and empirical studies support these predictions (reviewed in Clutton-Brock and Iason 1986; Wrensch and Ebert 1993; West and Sheldon 2002). This success primarily concerns invertebrates, especially groups with haplodiploid sex determination (Hamilton 1967; Charnov 1982) where sex ratio at birth can be controlled by whether the egg is fer-

tilized. Chromosomal sex determination was thought to constrain deviations from a 1:1 offspring sex ratio, but recent reviews have shown that this is not necessarily the case (e.g., West and Sheldon 2002). Less attention has been paid to adult sex ratio in vertebrates although Donald (2007) has shown a small but consistent male bias in birds and female bias in mammals.

Understanding variation in adult sex ratio is especially important because unbalanced sex ratios are one of the drivers of sexual selection (Kvarnemo and Ahnesjö 1996; Clutton-Brock 2007), affect mating system (Emlen and Oring 1977), cause increased aggression in the more common sex while altering mate choice in the less common sex (Krupa and Sih 1993; Jirotkul 1999), and



influence population persistence and conservation status because of the effect of sex ratio on effective population size (Donald 2007). Several mechanisms can result in a biased adult sex ratio (reviews in Gibbons 1990; Donald 2007). First, offspring sex ratios may persist into adulthood. Second, there may be a sampling bias, which will erroneously make one sex appear more abundant than the other. Third, sex differences in mortality will result in an excess of the sex with greater survival. Fourth, sexes may differ in their migratory tendency, although this will only affect the sex ratio if immigration and emigration are not balanced. Fifth, sexes may differ in their age at maturity resulting in an excess of the sex that matures earlier. Finally, sexes may differ in adult life span such that the longer lived sex will eventually become the more abundant. Although life span is often correlated with mortality rate, this need not be the case if mortality rate varies with age, especially if age-dependent mortality differs between the sexes. There appear to be very few vertebrate studies that attempt to estimate what combination of mechanism generates bias in adult sex ratio (see, e.g., Schmutz et al. 1979; Gibbons 1990; Hailey 1990; Lovich and Gibbons 1990). More often, studies examine single factors (e.g., Britton and Moser 1982 and references therein; Alho et al. 2008; Stenzel et al. 2011) and some have determined at what stage of life a biased sex ratio appears (e.g., Awkerman et al. 2007; Maness et al. 2007).

The Trinidadian guppy (*Poecilia reticulata*) is a good subject for studying sex-ratio variation at many levels. The guppy is a small live-bearing fish native to mountain streams of Trinidad. It is sexually dimorphic with males being much smaller than females and showing highly variable color patterns that are known to be under strong sexual selection (Endler and Houde 1995; Jirotkul 1999; Brooks and Endler 2001; Lindholm and Breden 2002). Variation in predation pressure has long been invoked as a key factor influencing the evolution of life history (Reznick and Bryga 1996; Dowdall et al. 2012), morphology (Hendry et al. 2006; Burns et al. 2009), and behavior (Seghers 1974; Luyten and Liley 1985; Magurran and Seghers 1990) in this species but it has recently become clear that other factors including local productivity (Grether et al. 2001; Bashey 2008; Zandona et al. 2011) and population density (Reznick et al. 2012) may be as important. Several investigators have shown that populations typically show a female-biased adult sex ratio (Seghers 1973; Rodd and Reznick 1997; Pettersson et al. 2004; McKellar et al. 2009). Preferential predation of the killifish *Rivulus hartii* on male guppies has been invoked as one likely cause of greater numbers of female guppies in the adult population (Seghers 1973; McKellar et al. 2009) although the evidence remains circumstantial. In addition, Pettersson et al. (2004) showed that sex ratio within four populations varied dramatically throughout the year, deviating significantly from 1:1 in only about a quarter of months. Censusing a population once or twice may, therefore, not give an accurate picture of typical

sex ratios within populations, but censusing methods themselves might also be a source of variation. Here, we take advantage of a long-term mark-recapture study in four introduction populations of guppies to examine how a female-biased sex ratio develops over time and how it fluctuates throughout the year. In addition, we can test which mechanisms contribute to the observed female-biased adult sex ratio. Because the sexes mature at the same age in guppies (Reznick and Endler 1982; Reznick and Bryga 1987), we do not consider age at maturation. We do test for sex differences in offspring sex ratio, catchability, life span, and the combined effect of mortality and migration. We also test for the relative importance of all these factors in influencing variation in adult sex ratio.

Methods

Our study populations were established in four headwater streams of the Guanapo River that originally had no guppies and only one resident species of fish (*R. hartii*). The introduced guppies were derived from a single locality on the Guanapo River where guppies coexist with a variety of predators. We have not measured sex ratio in this population, however Seghers (1973) found a female-biased sex ratio ($0.42 = 331 \text{ males}/780 \text{ mature fish}$) when he sampled a similar population in this river in 1969. The introduced guppies were collected from the wild as juveniles. The juveniles were raised to maturity in single sex groups and then mated prior to introduction into the transplant locales. In March of 2008, equal numbers of male and female guppies were transplanted into two upstream locations on the same river, 37 of each sex into Upper La Laja (UL) and 38 of each sex into Lower La Laja (LL). In March of 2009, a similar transplant from the same source was made into two additional guppy-free locales, 51 of each sex into Taylor (TY) and 64 of each sex into Caigual (CA). These locations are typical of low-predation habitat in being small, well-shaded streams containing no other fish except *R. hartii*. The introduction sites are located between barrier waterfalls preventing immigration and severely limiting emigration. The tree canopy was thinned from UL and TY allowing additional sunlight to reach the water increasing local productivity (Kohler et al. 2012) and somatic condition of females (A. Lopez-Sepulcre, unpubl. ms.). McKellar et al. (2009) found that the proportion of male guppies increased with more open canopies in natural guppy populations. Because reproductive success in male guppies shows greater variance and skew than in female guppies (Becher and Magurran 2004), females in good condition are expected to produce male-biased broods (Trivers and Willard 1973). If this prediction is true, then we predict a more female-biased sex ratio in unmanipulated populations compared to the more productive populations with thinned canopies similar to McKellar et al. (2009).

Each fish at a given population was given a unique mark by injecting colored elastomer (Northwest Marine Technologies) just below the skin. We used 12 colors with eight possible

marking positions on the body and two marks per fish. Populations were resampled each month and each unmarked fish that was at least 14 mm in standard length was given a unique mark. At this size fish can be sexed reliably but they are not fully mature. Thus we treat new recruits as indicative of juvenile sex ratio just prior to maturation. Fish that were recaptured (i.e., had already been marked) were all fully mature and thus treated as the adult population. Sex ratio in each case was estimated as percentage of males ($= N_{\text{male}} / (N_{\text{male}} + N_{\text{female}})$). Data from initiation of populations through December 2012 are presented here (58 months for LL and UL, 46 months for CA and TY).

We first tested whether adult (previously marked) populations differed from a 1:1 sex ratio for each month. Because all four adult populations showed a female-biased sex ratio nearly every month (see results), we next tested four hypotheses that might generate this bias: (1) a female bias in recruitment of adults, (2) sex differences in catchability, (3) greater mortality rates in mature male fish than female fish, and (4) a shorter life span in male fish than in female fish. Although we might expect mortality rate and life span to be correlated, these factors were independent here because of monthly heterogeneity in age-specific mortality. For each month a given fish can be present and caught, present but missed in the current census (in which case it was caught at a later time), or no longer present due to death or migration and thus never caught again. Migration between adjacent pools is typically less than 5% of the population (Reznick et al. 1996), and longer distance migration less than 1% (Bryant and Reznick 2004). Because we sampled the entire reach between barrier waterfalls, the only potential emigration is of fish washed over the downstream waterfall and no potential for immigration. As such, we treat all disappearances as deaths. Life spans were estimated as the average age of fish for each sex that died that particular month. Because we could not mark newborns, this is adult life span (from recruitment to death) rather than total life span (from birth to death).

We tested whether a bias in offspring sex ratio might persist into the adult stage by examining the sex ratio of new recruits. Although not a direct measure of offspring sex ratio, a significant bias in the sex ratio of new recruits would be consistent with this hypothesis whereas no bias in the sex ratio of recruits would mean offspring sex ratio could not explain adult bias. To confirm that sex ratio at recruitment reflects sex ratio at birth, we collected approximately 40 juvenile guppies from each of the four introduction populations and from the high predation source population in March 2013. We reared fish to maturity in single sex groups then mated them to produce the first laboratory-born generation of offspring. Eight offspring were saved from each litter and reared to an age of 25 days in an 8-l aquarium then scored for sex. Sex was determined as in prior studies (e.g., Reznick 1982) on the basis of the dispersion of melanophores at the base of the abdomen where

females will later develop a gravid spot. We determined the sex ratio for 16–19 broods from each of the five localities.

STATISTICAL ANALYSES

To estimate sex-specific mortality rates, catchability, recruitment, and population sizes, we used a Cormack–Jolly–Seber model for each sex and each population using the POPAN module in program MARK (White and Burnham 1999). The fully time-dependent model always had the most support (each sex in all four populations). This procedure provided maximum-likelihood estimates of each parameter for each month including standard errors. We estimated adult-only values by eliminating recruits from each month in the original design matrix.

We tested whether adult or recruitment sex ratios deviated from 1:1 at each census using a binomial test with sequential Bonferroni correction within each population. These results accorded well with the 95% confidence intervals generated from program MARK. Because sex ratios, mortality rate, and catchability are percentages, bounded by zero and one, we arcsine square-root transformed these values for further parametric tests. We first tested whether canopy thinning affected any of these factors or life span using a general linear model with population nested within canopy thinning. Because there was never any effect of canopy, this factor was not included in further analyses. Sex differences in mortality rate and catchability were tested using a general linear model with sex, population, month of year, and all interactions as independent fixed-effect factors. Population was treated as a fixed effect because these are experimentally created introductions rather than randomly selected natural populations and because we were interested in whether the four populations differed. Life span could not be transformed to a normal distribution so we tested for sex differences using a Wilcoxon signed rank test. We also examined the role of recruitment sex ratio, sex difference in mortality (male/female), and sex differences in life span (male/female) to adult sex ratio using a generalized linear model with a binomial link function (glm command in R). In this case, adult sex ratio was not transformed and a log transformation of other ratios was sufficient to produce normal distributions yielding a substantially lower AIC than a model with untransformed values ($\Delta\text{AIC} = 55$). Although life span itself is not normally distributed, the log-transformed ratio of the sexes was, so this factor did not require any special treatment in this analysis. Catchability was not included as a factor here because our estimate of adult numbers accounts for this.

A formal time series analysis indicated first-order autocorrelation in all variables with an additional peak at 12 months indicating seasonality. Differencing with a lag of one month removed this autocorrelation and the seasonal peak. Because statistical significance was the same whether we used undifferenced or differenced values, we report results on the undifferenced

values. The loss of the seasonal signal after differencing likely reflects the fact that the timing of wet and dry seasons varies from year to year. Sex ratio might still relate to wet and dry season even if this does not appear in the autocorrelation structure. We examined this possibility in two ways. First, if regional conditions (e.g., rainfall) influence sex ratio rather than just local effects (e.g., predator density), then we would expect month-to-month fluctuations in sex ratio to be correlated among populations. In addition, we had daily water level data for one stream, LL, from the time when guppies were first introduced in 2008 to September of 2010 minus a two-month gap, March and April of 2009, when a flood had washed out the data logger. We converted these data to monthly average water levels and correlated these values with adult sex ratio in LL with a 0–9 month lag.

Results

SEX-RATIO TRENDS

The canopy treatment had no effect on any factor (all $P > 0.33$), so this was removed from all further analyses. All four populations showed a female-biased adult sex ratio (Fig. 1). Because each population started with an equal sex ratio, it took from three to seven months for a significant female-biased sex ratio to become established using a binomial test. Once established, all populations remained significantly female biased (solid symbols in Fig. 1). The general linear model indicated a marginally significant difference among populations ($F_{3,196} = 2.63$; $P = 0.052$), likely due to the slightly less biased sex ratio for TY; removing this population eliminated this effect ($F_{2,153} = 0.32$; $P = 0.72$). In all populations, sex ratio became more female biased over time ($F_{1,196} = 27.61$; $P < 0.001$) with a similar rate of change in all four populations as the population-by-month interaction was not significant ($F_{3,196} = 0.36$; $P = 0.78$). In fact, monthly fluctuation in adult sex ratio was highly correlated among populations (Table 1).

Sex ratios of new recruits rarely differed significantly from 1:1 when analyzed on a monthly basis using binomial tests (solid symbols in Fig. 2). In a general linear model, the four populations showed no difference in recruitment sex ratio ($F_{3,190} = 0.59$; $P = 0.62$) nor did recruitment sex ratio vary over time ($F_{1,190} = 1.94$; $P = 0.17$), and there was no interaction between the two factors ($F_{3,190} = 0.33$; $P = 0.80$). There was also no correlation among populations in recruitment sex ratio on a monthly basis (Table 1). Although recruitment sex ratio did not differ significantly from 1:1, the majority of estimated ratios (140/198 = 70.7%) fall below the 50% line meaning females outnumber males (Fig. 2). When the entirety of the recruitment data (all populations and all months) was analyzed as a single sample, then the sex ratio of recruits did deviate significantly from 0.5 ($t_{199} = -7.01$; $P < 0.001$), averaging 0.470. Thus, although difficult to detect, there

does seem to be a small female bias in recruitment sex ratio. This bias may reflect differential male mortality prior to maturation or a female bias at birth. To test for sex ratio at birth, we collected juveniles from each population plus fish from the source population further downstream and reared them in the laboratory until maturity. We then mated these fish and collected offspring from 16 or 19 (LL only) females. We haphazardly selected eight young from each brood and reared them until sex could be determined. Overall, there was a significant female-biased sex ratio in newborns ($t_{82} = -2.27$; $P = 0.026$) with an average of 0.453, similar to what was seen in the wild. Interestingly, although sex ratios varied from 0.37 to 0.52, the natural, high predation population (Guanapo River) has the same sex ratio (0.46) as the average of the four introduction, low-predation populations (0.45). Thus sex ratio at birth does not seem to have changed during the four to five years since introduced populations were established.

Monthly recapture rates averaged 93% for all populations combined. The lowest recapture rate was 63% for females in LL during February 2009 when a flash flood curtailed sampling. Although fish were rarely missed, we did have a significant sampling bias ($F_{1,396} = 62.72$; $P < 0.001$) with an average of 8.8% of females being missed each month but only 5.4% of males being missed (Fig. 3). There was also a significant difference among populations ($F_{3,396} = 7.69$; $P < 0.001$) with the frequency of missed fish being lower in CA and TY than in UL or LL. Catchability also varied among months ($F_{1,396} = 16.49$; $P < 0.001$) with fewer fish being missed as the experiment progressed. The significant population-by-sex interaction ($F_{3,396} = 3.81$; $P = 0.010$) and population-by-month interaction $F_{3,396} = 3.33$; $P = 0.020$) are likely due to the lower number of females missed in CA.

CAUSE OF FEMALE-BIASED SEX RATIOS

Male mortality rate was greater than female mortality rate ($F_{1,398} = 68.645$; $P < 0.001$). Mortality rate also varied among populations ($F_{3,398} = 44.773$; $P < 0.001$) and although the main effect of month was not significant ($F_{1,398} = 0.602$; $P = 0.439$), there was a significant interaction between population and month ($F_{3,398} = 25.067$; $P < 0.001$). This effect appears to be due to the very high mortality for both sexes in TY for the first two years following initiation of this population (Fig. 4). As a result, male mortality averaged 2.3 times that of females in the other populations but only 1.5 times that of females in TY.

Life span showed a nonlinear relationship with month of the experiment so we analyzed these data using a nonparametric Wilcoxon signed rank test for each population. Female life span was significantly longer than male life span in all four cases (Fig. 5) but the difference in life span was much less for TY (LL: mean difference = 2.72 months, $Z = 6.134$, $P < 0.001$; UL: mean difference = 2.79 months, $Z = 6.359$, $P < 0.001$; CA:

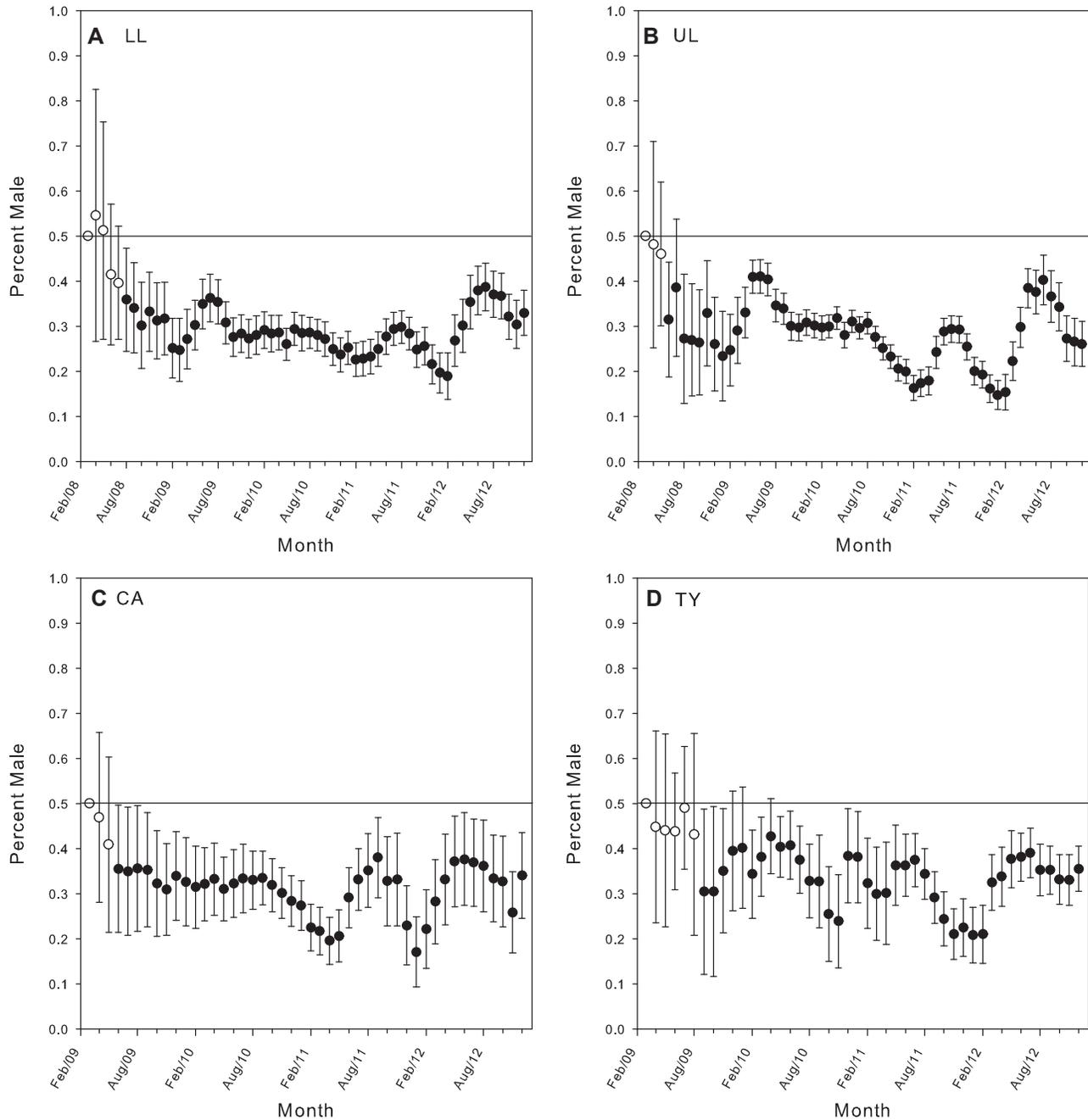


Figure 1. Adult sex ratio (percent male) and 95% confidence intervals derived from maximum-likelihood estimate of ratios. Solid symbols are significantly different from an equal sex ratio (solid line at 0.5) using a binomial test with sequential Bonferroni correction. Open symbols were not significantly different from equal sex ratio.

mean difference = 1.75 months, $Z = 5.240$, $P < 0.001$; TY: mean difference = 0.92 months, $Z = 5.120$, $P < 0.001$).

Next, we examined the joint effects of recruitment, mortality, and life span for explaining the female-biased sex ratio. A preliminary analysis indicated a strong interaction between population and all other independent factors (all $P < 0.001$) so we analyzed populations separately. In all four populations,

mortality rate and life span both contributed strongly to adult sex ratio. In the two younger populations (CA and TY), recruitment ratio also contributed significantly to adult sex ratio (Table 2).

Adult sex ratio was not correlated with water level in LL for the same month ($r = 0.034$; $P = 0.88$). However, there was a strong correlation between water level and sex ratio six months

Table 1. Pairwise correlations among populations in sex ratio for adults (above the diagonal) and recruits (below the diagonal).

	LL	UL	CA	TY
LL	-	0.869	0.652	0.579
UL	0.344	-	0.780	0.735
CA	0.061	0.070	-	0.524
TY	0.285	0.341	0.112	-

Values in bold were significant after Bonferroni correction.

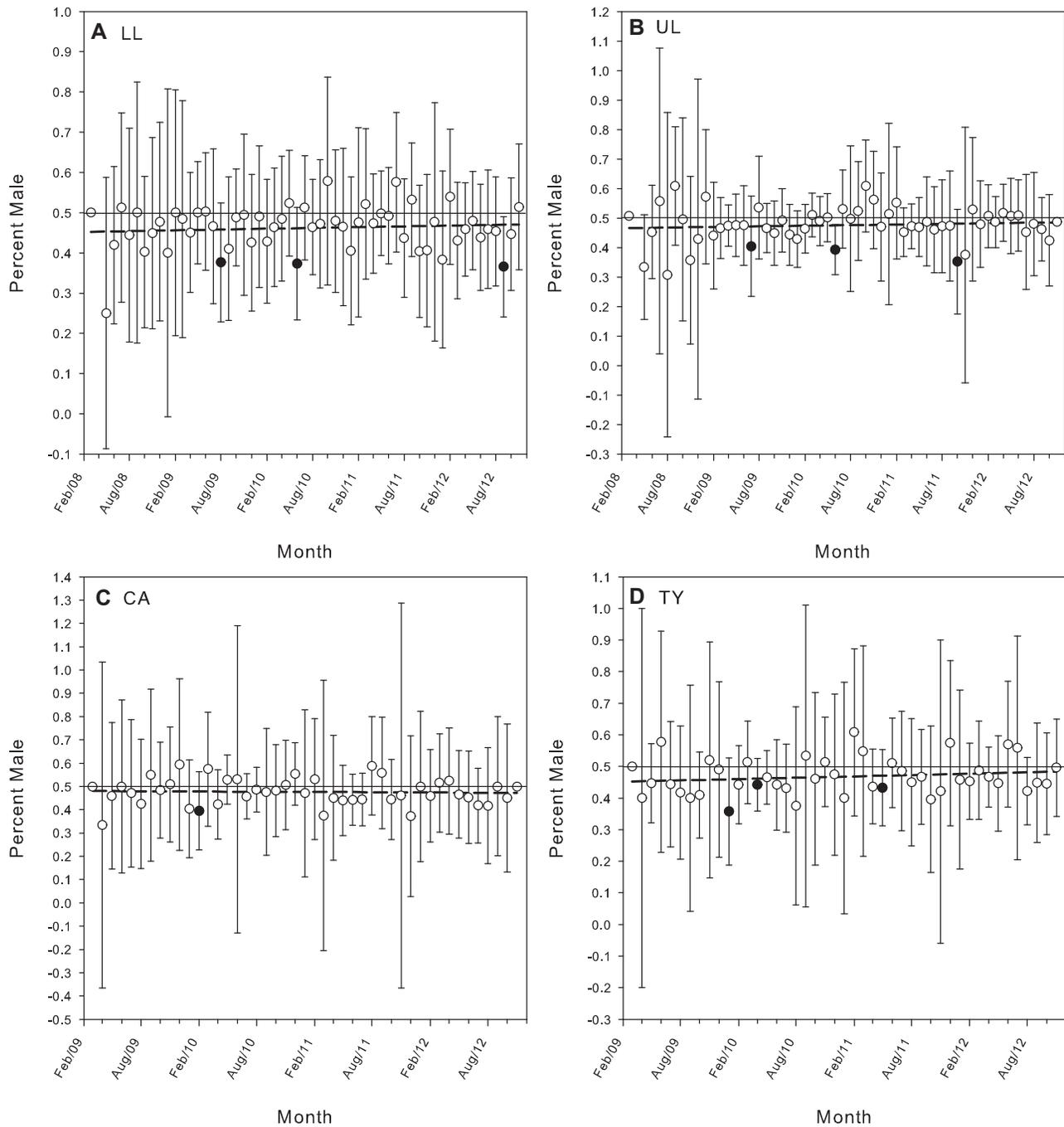


Figure 2. Sex ratio (percent male) of new recruits (subadults) with 95% confidence intervals. Solid symbols show months where the ratio differed significantly from an even sex ratio given by solid line, open symbols are not significantly different from even sex ratio. Dashed line shows the slight but significantly female-biased sex ratio when considering all months.

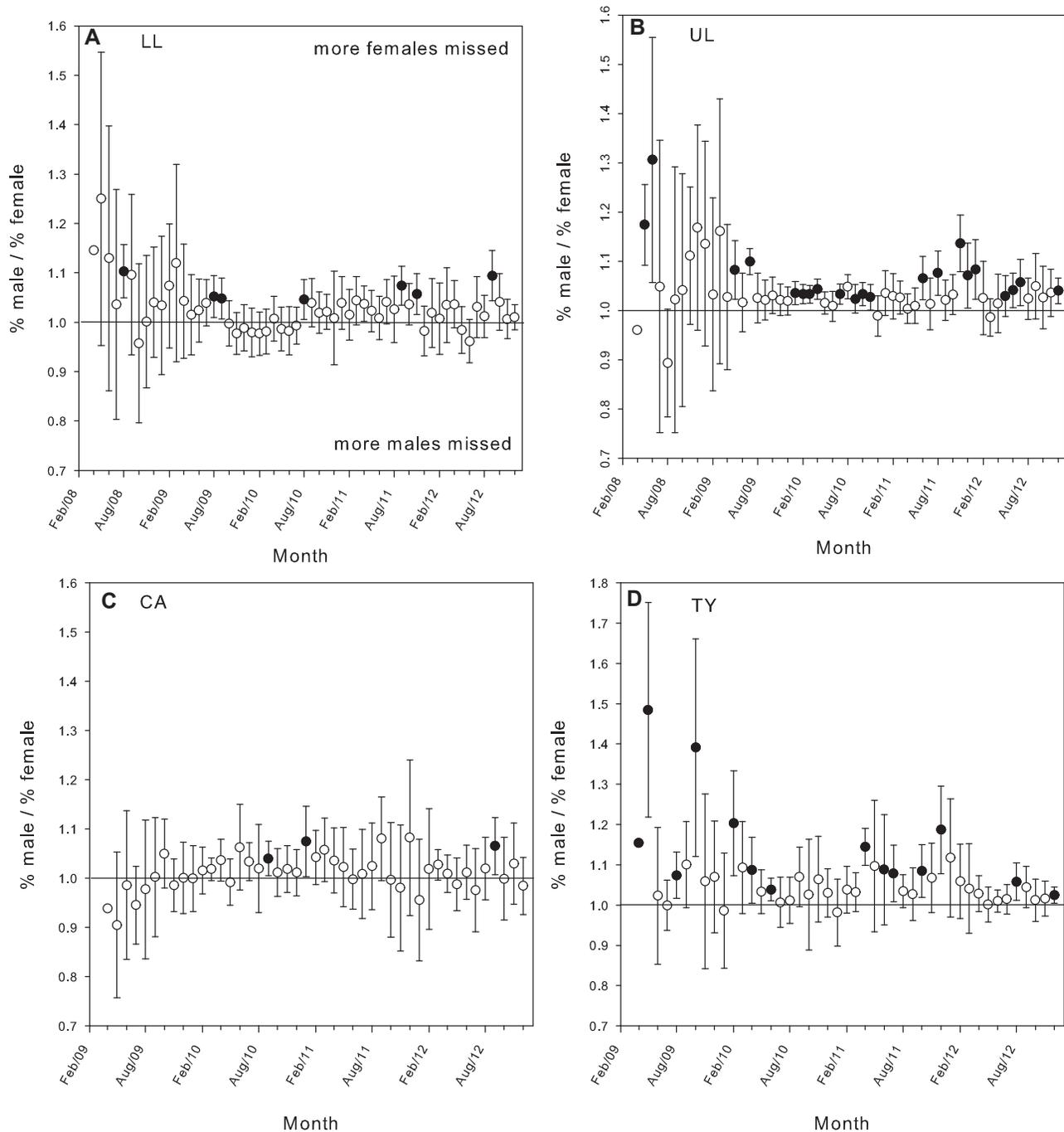


Figure 3. Ratio in catchability of males to females. Solid line at 1.0 is the line of equal catchability between the sexes. Points above this line indicate months where more females were missed than males, points below the line are months where more males were missed. Solid symbols indicate individual points where 95% confidence intervals do not overlap the equal catchability line.

later ($r = 0.755$; $P < 0.001$) with this lag time giving the strongest correlation. The next strongest correlations were for five months ($r = 0.661$; $P = 0.006$) and seven months ($r = 0.538$; $P = 0.14$) with no others being significant after Bonferroni correction. Sex ratio becomes less female-biased six months following high water levels (Fig. 6).

Discussion

We have shown that a strong female-biased adult sex ratio quickly emerges and is maintained for more than four years in newly colonized low-predation populations of guppies in Trinidad. We tested four potential factors that might explain this pattern: (1) a bias in the sex ratio of juveniles, (2) sex differences in catchability, (3)

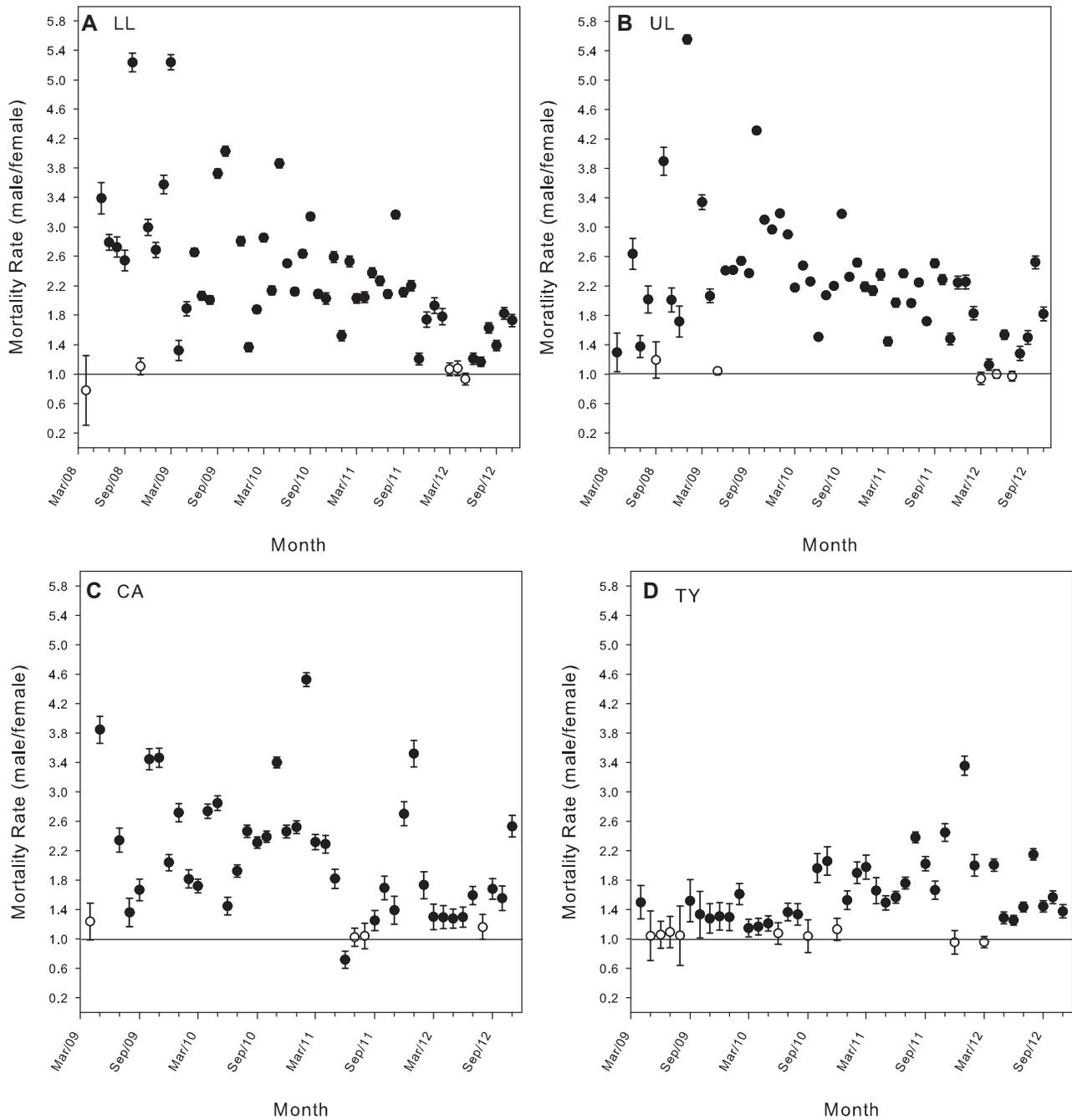


Figure 4. Ratio of mortality rates (male/female) with 95% confidence intervals. Solid symbols indicate individual points where 95% confidence intervals do not overlap the equal mortality line. In only one month was female mortality significantly greater than male mortality (June, 2011 in CA).

sex differences in mortality rates, and (4) sex differences in life span. Differences in age at maturation have also been shown to be critical in determining sex-ratio bias in some species (Hailey 1990; Lovich and Gibbons 1990), but sexes mature at the same age in guppies (Reznick and Endler 1982; Reznick and Bryga 1987) so this factor is not relevant here. Despite significant sex differences for all factors, the female-biased sex ratio was pri-

marily due to a longer female life span and higher male mortality in all four populations. Although the female bias in recruitment contributed significantly to adult sex ratio in two populations (CA and TY), this sex ratio (0.47 for both populations) is much smaller than the typical adult sex ratio (0.33 for both populations). Sex difference in catchability should not contribute to estimates of sex bias in mark-recapture studies and in any case was in the wrong

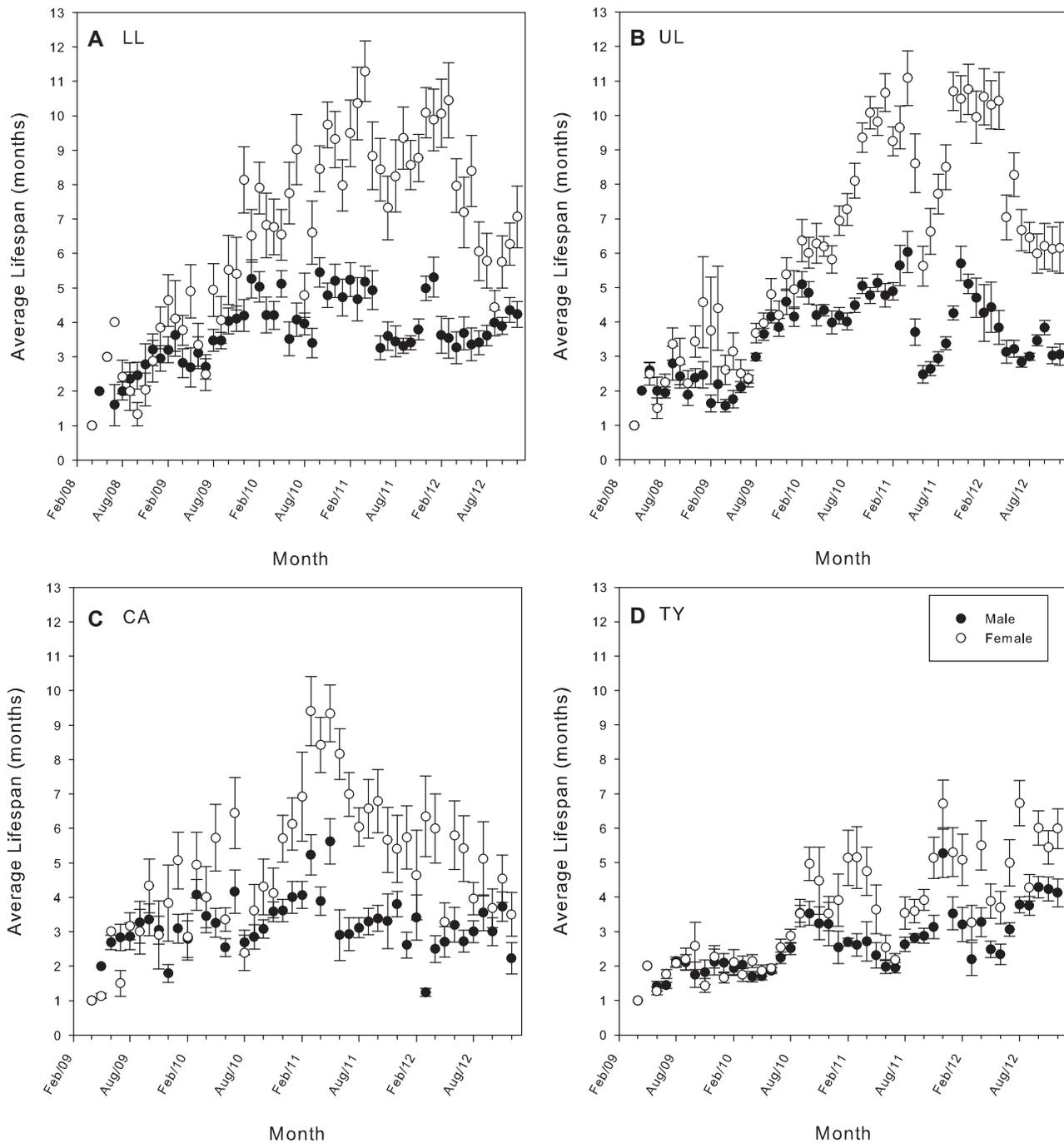


Figure 5. Average age at death for each month for males (solid line and symbol) and females (dotted line and open symbol). Error bars represent standard error on estimate of life span.

direction to explain the female bias seen here. The fact that there are typically more than twice as many female guppies as male guppies in these populations should result in strong sexual selection. However, sperm storage in guppies means that a male may continue to contribute to the reproductive pool long after it has died (Lopez-Sepulcre et al. 2013). Thus operational sex ratio is probably less biased than what we have found here but we cannot

determine this value until completing a pedigree analysis for all four populations.

Seghers (1973) and Pettersson et al. (2004) found no bias in offspring (secondary) sex ratio suggesting that offspring sex ratio varies little from 1:1 in the wild. We found only a minimal female-bias sex ratio of recruits (0.47 overall) and a similar bias in offspring sex ratio under laboratory conditions (0.45) for our

Table 2. Results of generalized linear regression on adult sex ratio using a binomial link function for each population.

Population	LL	UL	CA	TY
Intercept	-0.449 (0.104)***	-0.193 (0.082)*	-0.099 (0.158)	+0.368 (0.163)*
Recruit ratio	+0.329 (0.263)	+0.353 (0.211)	+1.058 (0.440)*	+2.184 (0.444)***
Mortality	-0.540 (0.079)***	-0.875 (0.076)***	-0.755 (0.129)***	-1.160 (0.179)***
Life span	+0.750 (0.098)***	+1.414 (0.086)***	+0.847 (0.164)***	+0.690 (0.255)**

Significant factors are given in bold (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Values represent coefficients (and std. error) of log-transformed ratios.

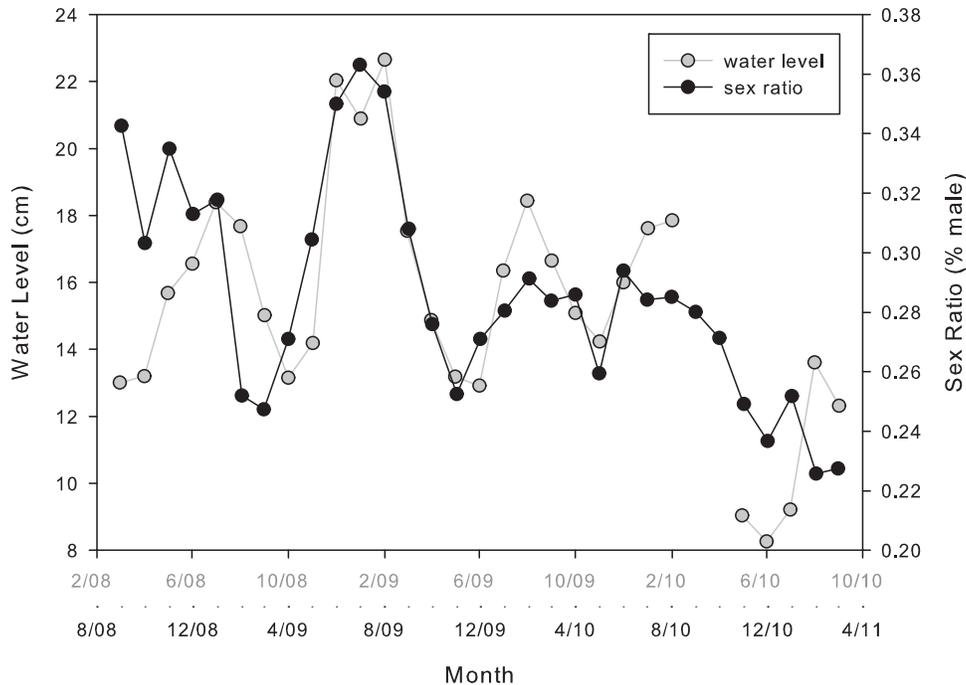


Figure 6. Relationship between average monthly water level (gray) and sex ratio (black) in LL from 2008 to 2010. The values for sex ratio (lower, black dates on x-axis) are displaced six months relative to water level (upper, gray dates on x-axis). Thus low water levels (i.e., the dry season) appear to be associated with a greater female bias in the sex ratio six months later. Missing values in 2010 were due to loss of water gauges following flash floods.

populations. Others have shown that offspring sex ratio can vary in guppies either due to genetic effects (Farr 1981; Watt et al. 2001) or phenotypic plasticity (Geodakyan et al. 1967; Karino et al. 2006; Sato and Karino 2010; but see Brown 1982), but the small bias we see in offspring sex ratio is not enough to explain adult sex ratio. We might also expect female condition to influence offspring sex ratio with females in good condition producing more male young. Canopy openness, and hence local productivity, correlated with adult sex ratio in McKellar et al. (2009), but we found no effect of canopy openness on either recruitment or adult sex ratio in our populations. In addition, local productivity depends upon season, becoming very low during the wet season (Kohler et al. 2012). If female condition affected offspring sex ratio then recruitment sex ratio should also vary with season for each population. However, we saw no correlation between

water level, as a proxy for season, and juvenile sex ratio (unpubl. data). Thus there appears to be no effect of local productivity on juvenile sex ratio at the population level in our study.

Sex differences in catchability may have a serious effect on our ability to determine sex ratio in the wild (Gibbons 1990). There were very few months when all fish were recaptured in our censuses, but we rarely missed more than 10% of the population. Because females were missed almost twice as frequently as males, our sampling bias favors males. Mark-recapture studies can incorporate missed fish into estimates of population size and sex-ratio estimates, so catchability does not affect our results. However, most studies of sex ratio in guppies have relied on single captures from populations (e.g., Seghers 1973; Rodd and Reznick 1997; Pettersson et al. 2004; McKellar et al. 2009) and thus will likely underestimate the degree of female bias actually present.

Our estimates indicate that female guppies are on average 1.65 times older than male guppies at death and that this difference is critical in explaining the female-biased sex ratio in all four populations. Given their longer life span, female guppies will accumulate within each population and may explain why female bias continues to increase in all four populations, although this appears to be approaching equilibrium. On average, male mortality was 2.1 times greater than female mortality and also contributed to sex-ratio variation in all four populations. Why do male guppies have higher mortality and shorter life spans than female guppies? In part, this might reflect higher migration rates in males rather than higher mortality. There is some indication that male guppies are more likely to migrate out of a pool than are female guppies (Croft et al. 2003). Our sites are bounded by barrier waterfalls and the major centers of populations are in pools far upstream of these waterfalls so we expect migration out of the study site to be minimal. In addition, studies in populations without barriers (Reznick et al. 1996; Bryant and Reznick 2004) indicate that migration out of individual pools rarely exceeds 5%. Even if all migrants were male, this level of migration would shift a sex ratio of 0.5 to just 0.475, thus mortality is likely much more important than migration. Others (Seghers 1973 and McKellar et al. 2009) have suggested that males are more vulnerable to predators, especially *R. hartii*, and that this is key in explaining female-biased sex ratios because this predator preferentially feeds on male guppies in the laboratory (McKellar and Hendry 2011). Predation likely contributes to male mortality but cannot explain why temporal variation in adult sex ratio is correlated among our populations. This variation is more likely due to environmental factors such as wet–dry season or flooding events. We showed that sex ratio in LL is strongly correlated with water level in LL, becoming less female-biased six months after high water levels. Currently, we do not know why there is such a long delay but it probably relates both to direct effects such as mortality during flooding (as suggested by Pettersson et al. 2004) and indirect effects such as lower overall recruitment during the wet season. Our data show no correlation between water level and male mortality ($r = 0.115$), female mortality ($r = -0.101$), or their ratio ($r = 0.088$) in LL so we favor recruitment as an explanation for a lagged response to water level. Flood events scour the environment of food resources (Kohler et al. 2012) and are associated with a decline in growth rate, somatic condition, and reproduction. Conversely, dry weather is associated with higher resource availability, higher individual and population growth rates, and higher rates of reproduction. The time lag between a weather event and recruitment would be mediated through the time required for resource availability to affect the rate of reproduction, then the time between when offspring are born and are recruited into the size class that can be marked. Because new fish are recruited at nearly a 1:1 sex ratio, a period of high recruitment would make the population sex

ratio less female biased. A period of low recruitment would result in a progressive increase in the female-biased sex ratio.

Previous studies of guppy sex ratio in the wild (Seghers 1973; Rodd and Reznick 1997; Pettersson et al. 2004; McKellar et al. 2009) found that most populations show a female-biased sex ratio but that ratios vary from 0.17 to 0.68% male. In addition, Pettersson et al. (2004) censused four populations on a monthly basis for one year. They found that sex ratio in these populations varied around 1:1 within these populations. By contrast, all four populations examined here produced a strong female bias that persisted every month for nearly four (CA and TY) or five (LL and UL) years. Our study differs in several ways from all of the previous ones. First, our populations are newly established and in the process of adapting to local conditions. It is possible that, when animals invade new habitats, a female-biased sex ratio will be favored as the population expands (Clutton-Brock and Iason 1986; Kocher 2006). However, this appears to depend upon peculiar sex-determining mechanisms (Clutton-Brock and Iason 1986; Lande et al. 2001) that do not occur in guppies. Moreover, the female-biased sex ratio persists in our populations despite their having reached carrying capacity. Because all adults that initiated our populations were derived from a single source, it is possible that we inadvertently included males that are genetically predisposed to produce female-biased broods (e.g., Farr 1981; Watt et al. 2001). However, such genotypes have never been found in wild populations and only seem to appear in laboratory stock after decades of inbreeding (Farr 1981). We also did not detect a strong female bias in either new recruits or in our laboratory samples of offspring. A more likely explanation for the discrepancy between our results and the repeated censuses in Pettersson et al. (2004) is that all four of our populations are small, headwater, low-predation populations, whereas Pettersson et al. (2004) had a much more heterogeneous sample including wide, low elevation rivers and high-predation populations. All previous studies have shown that the female bias in sex ratio tends to be less in high-predation populations (Seghers 1973; Rodd and Reznick 1997; Pettersson et al. 2004; McKellar et al. 2009). Our study also differs from all previous guppy studies in terms of the sampling method used. We sampled our populations intensively at each census attempting to catch all guppies present, averaging 679 fish per locality per month. Other studies sampled much less intensively resulting in smaller sample sizes (averages of 105 adults per population for Pettersson et al. [2004]; 112 adults for McKellar et al. [2009]; and 291 adults for Seghers [1973]). In addition, the mark-recapture method we used allowed us to account for fish that were present but not caught in a particular month. We found that females are missed more often than males at an average rate of 3–11% depending upon population. Given the intensity of our sampling, this should represent a minimum difference in catchability. The difference in sampling technique

and population type likely explains why our sex-ratio estimates differed significantly from 1:1 in all months once a bias had been established whereas previous studies typically found significant differences in just one-quarter (Pettersson et al. 2004) to one-half (Seghers 1973; McKellar et al. 2009) of all populations and ratios that varied among months (Pettersson et al. 2004).

Only a few systems have been sampled as intensively and for as many years as ours and in a fashion that would enable one to address differences in the catchability of the sexes. Soay sheep (*Ovis aries*) have been censused annually on St. Kilda Island, Scotland since 1955. In an analysis of 12 years from 1960 to 1989, Clutton-Brock et al. (1991) showed that adult sex ratio is often female biased but only at low population levels following years with high overwinter mortality, which affects males more than females. Offspring sex ratio hovers around 1:1 under all conditions (Lindström et al. 2002). Populations of red deer (*Cervus elaphus*) on the island of Rum, Scotland have been unmanaged since 1972. Annual censuses from this time through 1991 show a switch from male-biased to a consistent female-biased sex ratio (Clutton-Brock and Loneragan 1994) apparently due to greater mortality of males from starvation during harsh winters and high population densities (Clutton-Brock and Coulson 2002). Unlike Soay sheep, red deer do appear to adjust offspring sex ratio. Dominant hinds produce more male offspring, at least during favorable years (Kruuk et al. 1999). Obviously, if this affects adult sex ratio it would tend to make them less female biased. Sex ratio of Great Tits (*Parus major*) in Wytham Wood, Oxford was analyzed over 20 years (1964–1983) and never showed an adult sex bias (Clobert et al. 1988). However, a five-year study of two Estonian populations showed a consistent female-biased sex ratio due to higher mortality of males (Horak and Lebreton 1998). Ground finches (*Geospiza*) have been followed in the Galapagos for more than 30 years. A male-biased sex ratio can sometimes occur because males dominate scarce resources during dry years, but in most years populations show no sex bias (Grant and Grant 2002). Although our study includes just five years of data, guppies have a short generation time, about 1.74 per year in stable, low-predation environments (Reznick and Reznick 1997) and even shorter for expanding populations such as these. Thus this study is comparable to those cited above in terms of number of generations represented in the time series.

Several patterns of emerge from all of these studies that are worth further investigation. First, offspring sex ratio seems to play a very limited role in determining adult sex ratio (although the reverse may still be true). Second, adult mortality rates appear to be critical in determining sex ratio although differences in life span have not been examined very often. As was the case for guppies, the two need not be strongly correlated. Third, different populations within a species may show very different adult sex ratios (e.g., Clutton-Brock and Iason 1986; Horak and Lebreton

1998). Among long-term mark-recapture studies, ours is unique in that we have four replicated populations confirming that the patterns determined for one are consistent for most low-predation populations of guppies and hence can document the deterministic origins of sex-ratio bias, which include male mortality rate and female life span. Finally, although it is expected that biased sex ratios will impact sexual selection (Kvarnemo and Ahnesjö 1996; Clutton-Brock 2007), less attention has been paid to the possibility that because sexual selection results in sex differences in mortality (e.g., Promislow et al. 1994; Moore and Wilson 2002; Liker and Székely 2005) and life span (e.g., Gotthard et al. 2000), it is also likely to *create* biased sex ratios.

Others (Seghers 1973; McKellar et al. 2009) have argued that predation by *R. hartii* alone can explain the bias in sex ratio. Our results suggest that the explanation is more complex with variation in sex ratio driven by difference between the sexes in mortality and life span as well as temporal variation in the rate of recruitment. The fact that our study represents four experimental populations that converge on the same pattern and for the same reasons argues for the generality of our conclusions, at least for guppies. More generally, our study stands as a robust case study of the complexity of factors that determine adult sex ratios. It is likely that adult sex ratio for any species with a structured population will not have a unifactorial explanation given the number of demographic variables that ultimately contribute to sex ratio.

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