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LIFE-HISTORY EVOLUTION IN GUPPIES VIII: THE DEMOGRAPHICS OF DENSITY REGULATION IN GUPPIES (*POECILIA RETICULATA*)

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In prior research, we found the way guppy life histories evolve in response to living in environments with a high or low risk of predation is consistent with life-history theory that assumes no density dependence. We later found that guppies from high-predation environments experience higher mortality rates than those from low-predation environments, but the increased risk was evenly distributed across all age/size classes. Life-history theory that assumes density-independent population growth predicts that life histories will not evolve under such circumstances, yet we have shown with field introduction experiments that they do evolve. However, theory that incorporates density regulation predicts this pattern of mortality can result in the patterns of life-history evolution we had observed. Here we report on density manipulation experiments performed in populations of guppies from low-predation environments to ask whether natural populations normally experience density regulation and, if so, to characterize the short-term demographic changes that underlie density regulation. Our experiments reveal that these populations are density regulated. Decreased density resulted in higher juvenile growth, decreased juvenile mortality rates, and increased reproductive investment by adult females. Increased density causes reduced offspring size, decreased fat storage by adult females, and increased adult mortality.

KEY WORDS: **Density regulation, life-history evolution, population biology.**

Density regulation has had multiple lives in the disciplines of ecology and evolution. It played a prominent role in Darwin's "principle of divergence," which is the subset of the "struggle for existence" that can be attributed to intraspecific competition (Darwin 1859). Density regulation later emerged as one half of the one of the oldest empirical controversies in ecology, which is whether density-dependent factors play the dominant role in limiting the abundance of organisms (Andrewartha and Birch 1954; reviewed in Nicholson 1933; Murdoch 1994; Bassar et al. 2010a). Density regulation came into focus in evolutionary biology through theory for the evolution of early life histories and senescence, where it can act in concert with other agents of selection, such as extrinsic risk of mortality, to govern the predicted pathway of evolution (reviewed in Charlesworth and Leon 1976; Michod 1979; Charlesworth 1980; Charlesworth 1994; Brommer 2000; Roff 2008). Our goal is to bridge the gap between what we need to know to understand density regulation as an agent of selection and what we typically know about its incidence in nature in limiting the abundance of organisms.

EVOLUTION
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Early mathematical models of life-history evolution that examined how extrinsic sources of age-specific mortality (i.e., predators, disease) selected for the evolution of the life history were developed for idealized populations that experienced no density regulation (reviewed by Stearns 1976; Brommer 2000). This demographic theory superseded earlier verbal theory that proposed that much life-history variation reflected evolved responses to different intensities of density regulation operating through resource availability (e.g., Lack 1947; MacArthur and Wilson 1967; Lack 1968; Pianka 1970). Mathematical life-history theory later incorporated these ideas by examining evolution in response to extrinsic risk of mortality in regulated populations. These models specifically addressed how the effects of density on age-specific birth and death rates contributed to the evolution of different life histories (Charlesworth and Leon 1976; Michod 1979; Charlesworth 1980).

An interesting outcome of density-dependent models is that the predicted evolutionary response to the same extrinsic mortality risk depends on the precise action of density dependence. For example, in the absence of any density dependence, an increase in extrinsic mortality rate uniformly distributed across all age classes will not select for a change in how reproductive effort is distributed across age classes. Introducing density regulation does not change this result provided that all age classes respond to density to the same degree. However, if juveniles are more sensitive to density than adults, then a uniform increase in extrinsic mortality risk selects for increased reproductive effort in adults (Charlesworth 1994, p. 222). In general, these models reveal that if we are to understand how life histories will evolve in regulated populations, there is no escaping the need for knowing the demographic details through which density regulation is attained (Michod 1979; Charlesworth 1980; Abrams 1993).

Few empirical studies provide the demographic details necessary for understanding density regulation as an agent of selection, despite a very large ecological literature on density regulation (reviewed by Bassar et al. 2010a). The extensive analyses of time series of census data, typically long runs of numbers of adults, provide compelling evidence that some level of population regulation is widespread (e.g., Wolda and Dennis 1993; Sibley et al. 2005; Brook and Bradshaw 2006; Ziebarth et al. 2010) but reveal little about either the ecological agent of regulation or the underlying demographic mechanisms. Density perturbation experiments, in which the density of a natural population is either increased or decreased to see if it returns to premanipulation values (reviewed by Cappuccino and Harrison 1996), have been successful in demonstrating whether specific individual populations at ambient densities are experiencing density regulation. They have also been successful in delineating the ecological agent(s) of regulation, for example, discriminating between resource-based (bottom up) versus predator-based (top down) regulation. Most of these experiments, however, do not fully address the demographic mechanisms of density regulation, often because only one life stage was evaluated (Bassar et al. 2010a).

While there are some studies that provide the necessary information (Dobson and Oli 2001; Oli et al. 2001; Gustafsson and Ehrlen 2003; Fowler et al. 2006; MacDonald et al. 2009) (Dobson and Oli 2001; Oli et al. 2001), they represent only a small subset of such experiments. For example, Bassar et al. (2010a) found that only six of 28 density perturbation experiments collected enough data to allow a full life table response analysis, and only two of those six performed an explicit life table response analysis. Moreover, none of these studies were done in a context that would allow them to address the evolutionary consequences of density regulation.

Here we report on a replicated series of short-term density manipulations on natural populations of guppies. We wish to know if guppy populations normally experience density regulation and, if so, how regulation is manifested demographically. This study stands apart in our ability to focus the results on well-described patterns of life-history evolution.

BACKGROUND

In earlier research, we described how the life histories of guppies evolve in response to the risk of predation (Reznick 1982; Reznick and Endler 1982; Reznick and Bryga 1996). We have primarily compared guppies that live under low versus high risk of predation. Those that live under low risk are mostly found in headwater streams where they co-occur with one other fish species, *Rivulus hartii*, that is an occasional predator on guppies. When it feeds on guppies, it preys preferentially on small, immature size classes. Guppies that live under a high risk of predation are instead found in higher order streams where they co-occur with a diversity of predators, including the pike cichlid *Crenicichla alta*, the wolf fish *Hoplias malabaricus*, and the characin *Astyanax bimaculatus*. Some of these predators prey selectively on large, mature size classes of guppies. Guppies from low-predation environments have evolved delayed maturity and reduced reproductive effort relative to those that live under high risk of predation.

These differences in life histories among guppies from high- versus low-predation environments are repeated in many different watersheds (Reznick and Bryga 1996) and have a genetic basis (Reznick and Bryga 1996). Genetic data show that the life histories of the low-predation site guppies have evolved convergently in the separate drainages (Alexander et al. 2006). Furthermore, we have transplanted guppies from high-predation environments over barrier waterfalls that previously excluded all fish except *R. hartii*. The transplanted guppies evolved life histories typical of guppies from low-predation environments in 4–11 years (Reznick and Bryga 1987; Reznick et al. 1990; Reznick et al. 1997).

These results argue that predators play an important role in shaping guppy life-history evolution. The nature of the differences in guppy life histories are as one would predict from theory that models life-history evolution in idealized populations without any form of population regulation (Gadgil and Bossert 1970; Charlesworth 1980; Charlesworth 1994). However, some of our results are incompatible with this straightforward explanation. First, when we estimated size-specific mortality risk with mark-recapture studies, we expected to find relatively high adult mortality rates in high-predation environments and relatively high juvenile mortality rates in low-predation environments because of differences among predators in the sizes of guppies they prey upon. We instead found that the guppies from high-predation environments experienced uniformly higher mortality risk across all size classes than those in low-predation environments (Reznick et al. 1996). Theory that models life-history evolution without density regulation predicts that life-history differences will not evolve under such circumstances, yet we had already shown with transplantation experiments that they do evolve under those circumstances.

The patterns of life-history evolution that we have seen are compatible with life-history theory that invokes density regulation as an additional agent of selection through the indirect effects of predators on density (Reznick et al. 2001). That is, predators not only increase guppy mortality rates, they exert other effects through their lowering of prey population densities (Charlesworth 1994; Abrams and Rowe 1996). Guppies from high-predation communities are found at lower population densities and have higher individual growth rates than those from low-predation communities. The differences in fecundity in guppies from high- versus low-predation environments exceed what we see in the laboratory when guppies from both environments are reared on the same level of food availability (Reznick and Bryga 1996; Reznick et al. 1996). The higher fecundity and higher growth rates of guppies from high-predation environments are consistent with their having higher levels of food availability. Conversely, the higher population densities, lower growth rates, and lower fecundities of guppies in low-predation environments can also be explained by an expansion of guppy populations when predators are absent and mortality rates decline. Abrams and Rowe (1996) modeled the consequences of such indirect effects of predation and showed that our observed pattern of life-history evolution can be reconciled with the size-independent increase in mortality risk on high-predation environments.

These results raise the question of whether guppy populations are density regulated, particularly those that experience potential release from predator limitation and, if so, through which demographic responses to density. Here we report on the results of experiments in which we either increased or decreased the ambient density of guppies in low-predation environments, then compared their demographic responses to unmanipulated controls.

Methods

EXPERIMENTAL DESIGN

All experiments were performed in small, headwater streams on the southern slope of the Northern Range Mountains in Trinidad. We replicated the experiment seven times: twice in a tributary to the Arima River in 1993, twice in two tributaries to the Quare River in 1994 (Quare 2 and 6) (Endler 1978), twice in a third tributary to the Quare River in 1995 (Quare 7) (Endler 1978), and once in a tributary to the Aripo River in 1996. All streams had low-predation communities, meaning that only guppies and the killifish *R. hartii* were present. All streams had distinct riffle-pool structures. Guppies in such environments preferentially occupy pools and have low migration rates from one pool to the next; in our prior work we found that 4–5% of the fish emigrated during a 2-week period and that the émigrés were most likely to be mature males (Reznick et al. 1996). We treated individual pools as sampling units. We selected three pools, not necessarily adjacent to one another, that were comparable in size and structure. The selected pools tended to be bounded up- and downstream by distinct riffles or small waterfalls to enhance their isolation from other portions of the stream. We also often further enhanced the pools' isolation by inserting a sheet of nitex screening at the up- and downstream boundaries, held in place by gravel and stones taken from the natural substrate. All experiments were done during the dry season, when low water levels further enhance the isolation of pools.

We collected fish from the three pools with butterfly nets in a fashion that did not require that we enter the pool, so we did not modify the habitat in any way. Guppies are easily seen in the shallow, vegetation-less pools and are attracted to the sediment raised by maneuvering the net, which makes it possible to capture every guppy in a pool in a short interval of time. We revisited each pool repeatedly until we succeeded in catching all fish present. We kept the population from each pool in a separate bucket of medicated water, then brought them back to our field station.

At the field station, fish were lightly anaesthetized with MS-222, then measured for length under a dissecting scope and given a single mark with a subcutaneous injection of acrylic latex paint diluted in teleost ringer's solution. The marking scheme followed that of earlier studies (Reznick et al. 1996) and indicated the fish's millimeter size class. The size classes ranged from 12 to 26 mm, standard length. All mature males received the same mark, regardless of size. Males have determinate growth and hence did not grow during the course of the experiment. Our earlier research established that the paint marks are retained for >12 months and

that growth and survival are not affected by marking (Reznick et al. 1996). The marked and measured fish from all three pools were combined and placed in aerated holding tanks, with a separate tank for each size class.

In a subset of our experiments, we also marked newborn and small juveniles (6–12 mm, standard length). We did so by immersing them in a 250 mg/L solution of calcein (buffered to pH 8.0) for 24 h (Wilson et al. 1987). Calcein binds to calcium-bearing tissues and is later visible in the caudal fin rays, hypleural plate, and vertebrae when viewed through an epifluorescence microscope (following Rodd and Reznick 1991). In experiments on *Heterandria formosa*, another species in the family Poeciliidae, we demonstrated that calcein treatment does not affect survival, growth or age, and size at maturity. The mark remains visible in at least some tissues for 5 weeks or more (Leips et al. 2001).

We wanted the density treatments to be a manipulation that was indexed on the number and size distribution of fish in the pools at the start of the experiment. To do this, we enumerated the numbers of adult males, and of adult females and immature fish in each size class in all three pools and summarized the data in a table that had a row for each size class and a column for each of the three pools. We then used the row and column totals to generate the "expected" values for each pool so that each of them had the same size structure (Table 1). We used these expected values as the basis for determining the numbers of individuals of each sex and size class to reintroduce to the pools. We then assigned density treatments to the pools at random so that the number of guppies that were reintroduced would be half, equal, or twice the density of fish that were present in the pool before the experiment. Because the total numbers of fish required often exceeded those originally in the three pools, we supplemented the populations of the three experimental pools with fish collected from neighboring pools. The populations that were reintroduced into each pool were drawn at random from the holding tanks, where the populations of the three pools were combined but the fish were separated by size class. This process means that each introduced population was a haphazard mixture of the three original populations.

Our implicit assumption in choosing this design was that the size structures were equal in all three pools prior to the manipulation and that all pools were at the same density relative to their respective carrying capacities. The observed size structures did sometimes differ among pools. When this occurred, a consequence of our assuming that they did not differ would be a mismatch between the numbers and biomasses of fish introduced as a consequence of adjustments in the size structure of the population. Such differences between the original and introduced populations will contribute to residual variation and reduce the power of our statistical evaluation of the effects of our density manipulations. One virtue of assuming a similar size distribution in all treatments is that we gain proportional knowledge of the impact of density on all size classes.

We recollected all fish after 20–23 days. The duration of the manipulation was in part dictated by the limited duration of the field trip or other logistical constraints, but was also gauged to be less than one interbrood interval; guppies are livebearers that give birth approximately every 24–25 days in these habitat types. This duration means that all but the most advanced developing young in all pregnant females would have initiated development during the course of the experiment. We examined the fish for marks immediately after capture, measured their standard lengths, sorted them into their initial size classes, as indicated by their marks, then preserved all paint-marked fish in 5% formaldehyde for later examination. All small (<18 mm) unmarked fish were instead preserved in 70% ethanol, which preserves the calcein label.

DEPENDENT VARIABLES

Growth

Growth was estimated as the average growth increment for all individuals in each millimeter size class. We subtracted the mean size, averaged across all three pools, for a given size class prior to the start of the experiment from the mean size for that size class in a given pool upon recapture to estimate the growth increment for that size class. There was some level of imprecision associated with using the grand mean for a given millimeter size class, rather than the mean size for the fish that were introduced into each pool, the consequences of which would be increased residual variance and reduced statistical power.

Fat content

All individuals preserved at the end of the experiment were later dissected. The contents of the digestive tract were removed, the entire carcass was dried at 55°C overnight, stored in a desiccator, then weighed to the nearest 0.1 mg on an electrobalance. The carcasses were then extracted with anhydrous ether to remove triglycerides, redried, and reweighed. The percent fat in the initial dry weight was estimated from the weight lost as [1 − (lean dry weight/total dry weight)] *100.

Reproduction

If the fish was an adult female, we removed the ovary for separate treatment before drying the somatic tissues. We removed all developing embryos, recorded their stage of development as in previous studies (Reznick and Endler 1982), then dried and weighed the embryos separately. We then extracted the embryos with anhydrous ether and reweighed them so that we could estimate both mean embryo dry weight and embryo fat content. We weighed the other ovarian tissues separately and summed them with the mass of the embryos to yield the reproductive dry

Table 1. **An example of the pre- and postmanipulation population numbers for three pools for one replicate of this density manipulation experiment. The columns indicate the numbers of fish in each size class collected from each pool before the experiment, then introduced into the pool at the start of the experiment. The number introduced in each size class was the expected value {[(row total) x (column total)]/grand total} times the treatment value for that pool (halved density [1/2×], control [1×], or doubled density [2×]). These numbers correspond to the replicate run in a tributary to the Aripo River in 1996. In this example, the density of Pool 1 was reduced, the density of Pool 3 was increased, and Pool 2 was the control.**

Size	POOL 1 Observed	1/2x Introduced	POOL 2 Observed	CONTROL Introduced	POOL 3 Observed	2x Introduced
<12 mm	49	28	16	18	42	67
12–13 mm	19	10	8	7	12	24
14–15 mm	34	14	4	9	16	34
16–17 mm	28	12	5	8	13	29
18–19 mm	21	9	8	6	7	22
20–21 mm	2	3	4	2	4	6
>22 mm	1	2	5	1	1	4
Males	26	13	10	9	14	31

mass. We then combined the somatic and reproductive tissue dry mass measurements to estimate a female's reproductive allocation (RA), or the percentage of the total dry mass that consists of developing embryos [reproductive dry mass/(somatic dry mass + reproductive dry mass)].

Survival

We estimated survival as the probability of recapture. We observed a few marked individuals that had emigrated out of the pool they were released in, but we did not systematically search for émigrés. Not including émigrés means that our estimates of survival tend to be underestimates of true survival.

STATISTICAL ANALYSES

We tested the effects of the density treatments on somatic growth rates, offspring size, RA, and adult fat content using a linear mixed model approach. Number of offspring and survival were modeled using a generalized linear mixed model (GLMM). Number of offspring was analyzed with log-link functions and Poisson error distributions. Survival of the different size classes was modeled with logit transformations and binomial error distributions. Because we were interested in testing whether the effect of the density treatment was different across size classes, we included either initial length (in growth and survival analyses) or final length (all others) and their interactions with density as fixed covariates. We also included a quadratic effect of initial length in the analyses of growth because it yielded a better fit than a linear relationship alone. We included the stage of development of offspring as a covariate in the offspring size analysis because offspring dry mass decreases through development (Reznick and Endler 1982). Only females with broods with stages less than 35 of 50 (Reznick 1981) were analyzed for number and size of offspring and RA because our manipulations were slightly less than one reproductive cycle of guppies. Doing so ensured that the offspring were initiated during the experiment.

For the female survival analysis, initial length was entered as a fixed categorical effect with five levels (juveniles [<12 mm], 12–14 mm, 14–18 mm, 18–22 mm, >22 mm) because we were unable to adequately capture variation among size classes using linear or polynomial fits when length was entered as a covariate. Analyzing survival of discrete size classes also provides comparability with previous studies of survival in wild guppy populations (Reznick et al. 1996). Males that were mature at the beginning of the experiment were analyzed with treatment entered as a fixed categorical effect.

We used individual fish as datapoints in all analyses. We included density treatment as a categorical fixed effect and entered stream and interactions between stream and density as categorical random effects. For two of the streams, we conducted two separate sets of replicate manipulations; we included these in all analyses as replicate nested within stream and replicate nested within the interaction between stream and density as categorical random effects. This random effect structure accounts for the hierarchical nature of the data when using individuals as datapoints. We used the between-within degrees of freedom method to calculate the degrees of freedom for tests of fixed effect parameters. For example, the proper degrees of freedom (and error variance) for tests of the density treatments is given by the numerator degrees of freedom for the interaction between density and stream under a standard general linear model or analysis of variance framework. When length covariates were included in the models, we also incorporated random interactions of these covariates with each of the random, categorical effects when estimable. Finally, because levels of the density treatment have by

definition different numbers of individual fish for each replicate, we used a separate error variance term for each level of the density treatment.

Our model fitting approach was to: (1) fit the full model (all fixed effects, their interactions, random effects, and separate error variances) with restricted maximum likelihood (REML) based methods, (2) fit the full model without separate error variances for each treatment, and (3) test the differences in the model fit using a likelihood ratio test with 2 degrees of freedom. We chose to use separate error variance terms because the likelihood ratio tests indicated that models were different enough to warrant separate variances for each density treatment. (4) We used *Z*-tests to decide whether to remove random covariate interactions (all were removed in every case). (5) We next used ML-based estimation methods to decide whether to remove fixed effect parameters (covariates and covariate interactions with density) using backwards selection. We removed these effects if the *P*-values from the omnibus *F*-tests were greater than 0.05. In some cases, some random effects were not estimable, so they were removed from the models.

We used planned comparisons to test the effect of increased and decreased density on each of the dependent variables. When significant interactions between size covariates and density treatment were present in the model (somatic growth only), we centered the data on three locations along the size covariate representing small (14 mm), medium (18 mm), and large (22 mm) individuals and tested the density effects at each of these locations. When there was a significant interaction between density treatment and stream (somatic growth only), we constructed contrasts for each stream replicate by incorporating the best linear unbiased predictor of the interaction between treatment and stream to yield narrow sense contrasts. RA and adult fat were arcsin-square-root transformed to normalize the residuals. Weekly somatic growth was calculated as (final length − initial length)/weeks of experiment because each stream replicate was of slightly different duration.

Results

GROWTH

There was a significant impact of density on growth in all replicates; reduced density tended to cause increased individual growth rates while increased density tended to cause reduced individual growth rates. However, the details of the responses differed among size classes (Table 2, Fig. 1). Density effects were always most consistent and pronounced in the smallest size classes, which are immature. The effects of density manipulations were somewhat lower in the middle size class, which spans the age and size at maturity, and disappear and may even be reversed in the largest size class, which consists exclusively of large, adult females. This change in the effect of density with size is correlated with a deceleration in growth as size increases. It is also associated with a change in how surplus resources are used by the different size classes. For juveniles, all surplus or deficits are reflected in somatic growth rate. For adults, changes in resource acquisition also influence allocation to fat reserves and reproduction (see below).

Table 2. ***t*-statistics from contrasts of decreased and increased density compared to controls for somatic growth. Contrasts for each stream are shown and were calculated using the narrow sense random effect of stream x density from the linear mixed model. Contrasts are also centered at 14 mm, 18 mm, and 22 mm sizes to show differences in the growth response at these sizes. Subscripts are degrees of freedom.**

Contrast	Arima	Aripo	Quare 2	Quare 6	Quare 7
Decrease (14 mm)	-0.315_8	-1.992_8	0.207_8	$2.872_{8}{}^{*}$	1.702_8
Increase (14 mm)	$-7.553_{8}{}^{*}$	$-6.180_{8}{}^{*}$	$-5.600_{8}{}^{*}$	-0.231_8	0.046_8
Decrease (18 mm)	$2.177_{8}{}^{**}$	0.290_8	$1.871_{8}{}^{**}$	$5.440_{8}{}^{*}$	$4.317_{8}{}^{*}$
Increase (18 mm)	$-6.951_{8}{}^{*}$	$-6.271_{8}{}^{*}$	$-5.403_{8}{}^{*}$	-0.521_8	-0.362_8
Decrease (22 mm)	-2.211_8	-3.331_8	-1.318_8	0.220_8	-0.942_8
Increase (22 mm)	$-5.028_{8}{}^{*}$	$-4.785_{8}{}^{*}$	$-4.599_{8}{}^{*}$	0.056_8	0.328_8

******P* **< 0.05; *****P* **< 0.10.**

There were also differences among replicates in the details of the response to density (Table 2, Fig. 1). Here we focus on the response of just the two smaller size classes (14 and 18 mm). In the Arima, Aripo, and Quare 2 experiments, the growth increments for the ambient and decreased density treatments were not different from one another, but increased population density caused a significant reduction in growth rate. In the Quare 6 and 7 experiments, the growth rates at increased and ambient densities were not different, but reduced population density caused an acceleration in growth rate.

The actual values for the growth increments suggest that these differences among replicates in density response are attributable to differences among streams in how close their populations were to carrying capacity prior to the manipulation. The growth increments for the control treatment in the Quare 6 and 7 replicates were half of those in the controls of Arima, Aripo, and Quare 2 replicates (Fig. 1—note different scales on the *y*-axis), which in turn suggests that the former two were more severely resource limited than the latter three at the outset of the experiment. The former two showed no further suppression of growth when density

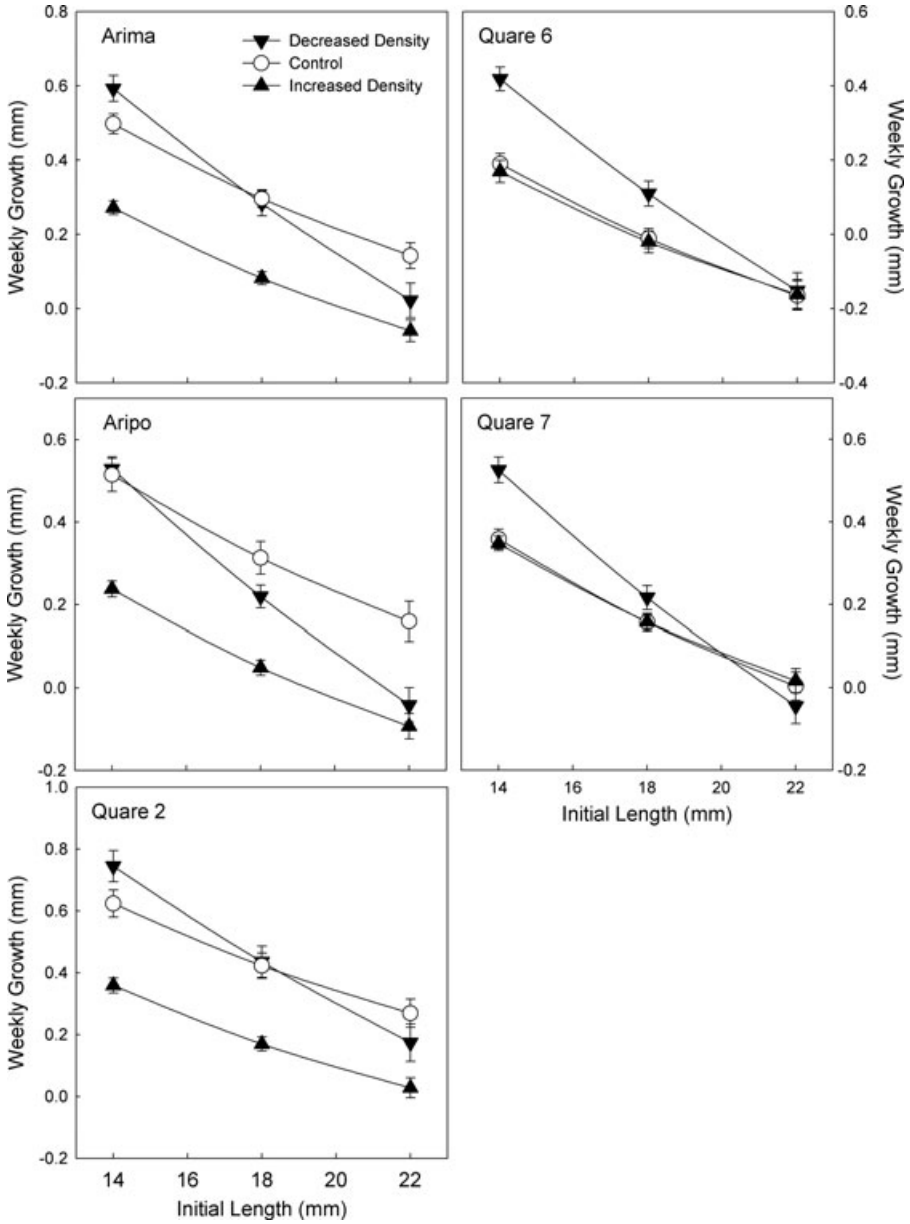

Figure 1. **Predicted means and standard errors from linear mixed model analysis of somatic growth. Analyses were conducted using length as a covariate. We illustrate the predicted means for three size classes that correspond to the three levels of the covariate. There were significant interactions between size covariates and density treatment so we centered the data on these three locations and tested the density effects at each of these locations. The illustrated values thus correspond to statistical tests at these covariate levels. Error bars are ± 1 standard error.**

was increased, but growth accelerated when density was reduced, presumably because the population density was now well below carrying capacity. The latter three, with higher growth rates in the controls, were presumably less resource limited at the outset. There was no further acceleration of growth when density was reduced, but there was a significant reduction in growth when density was increased, presumably because the populations were now sufficiently dense for resource limitation to restrict growth rate.

Table 3. ***t*-statistics from contrasts of individuals from pools with decreased or increased density compared with control treatments. Subscripts are degrees of freedom.**

Contrast	Female Fat	Offspring Size	Offspring Number	RA
Decrease	-0.177_8	0.496_8	1.556_8**	2.220_8*
Increase	-2.809_8*	-2.359_8*	-0.405_8	-0.893_8

*$P < 0.05$; **$P < 0.10$.

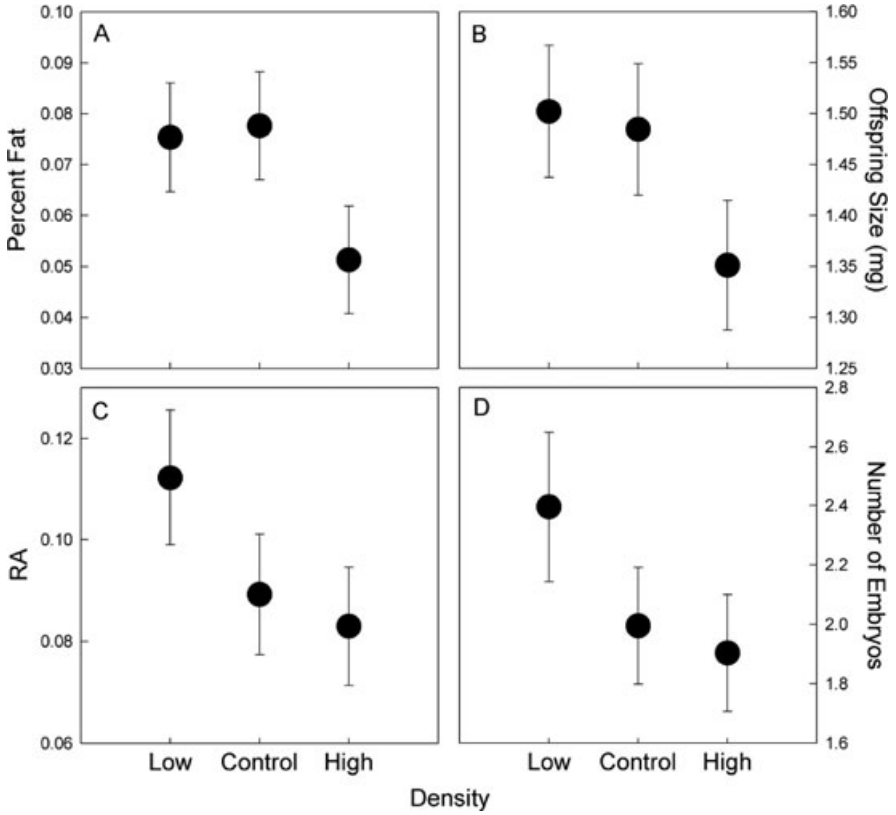

Figure 2. Least-square means from linear mixed model analyses of (A) proportion of female adult body mass from fat, (B) size of developing offspring, (C) proportion of adult body mass devoted to developing offspring (RA), (D) number of developing offspring. Error bars are ± 1 standard error.

ADULT FAT

We analyzed the fat content of adult females only. For this and all remaining dependent variables, we did not see any significant interactions between replicate and density treatment, so we are able to summarize the results in terms of the effects of treatment alone. There was no difference in the quantity of fat stores between the control or decreased density treatments (Table 3, Fig. 2). Adult females in both of those treatment groups had significantly greater fat reserves than the females in the increased density treatment.

REPRODUCTIVE ALLOCATION

The proportion of body weight that consisted of developing embryos (RA) was higher in females from the reduced density treatment ($P = 0.029$; Table 3, Fig. 2). There was not a significant difference between the control density and the increased density treatments.

The remaining two variables address how RA was divided between the number and size of the offspring, because these are the two components of RA.

SIZE AND NUMBER OF OFFSPRING

Females from the reduced density and control treatments produced significantly larger offspring than their counterparts from the increased density treatment (Table 3, Fig. 2). Females from the reduced density treatment tended to produce more offspring ($P = 0.08$) than those from the control or increased density treatment (Table 3, Fig. 2). The higher RA for fish in the reduced density treatment is thus the consequence of their producing larger and the tendency to produce more offspring per brood relative to females from the increased density treatment.

JUVENILE AND ADULT MORTALITY

Adult mortality rates increased significantly in pools with increased population density but were unchanged in pools with decreased population density (Table 4, Fig. 3). Because we did not search for émigrés, this increase could be due to increased emigration as well as higher death rates. Juvenile mortality rates declined significantly in pools with reduced population density but were unchanged in pools with increased population density.

Discussion

When we experimentally increased or decreased ambient population density, we observed changes in demography that would tend to return the populations back to ambient density. On the basis of these short-term responses, we conclude that natural populations

Table 4. ***t*-statistics from contrasts of decreased and increased density compared to controls for survival. Subscripts are degrees of freedom.**

Label	Babies (<12 mm)	12-14 mm	14-18 mm	18-22 mm	>22 mm	Adult Males
Decrease	2.409_{23}*	1.427_{23}	0.142_{23}	-1.123_{23}	-0.246_{23}	-0.618_{8}
Increase	−1.18423	-0.197_{23}	0.166_{23}	-1.809_{23}**	-2.855_{23}*	-0.867_{8}

*$P < 0.05$; **$P < 0.10$.

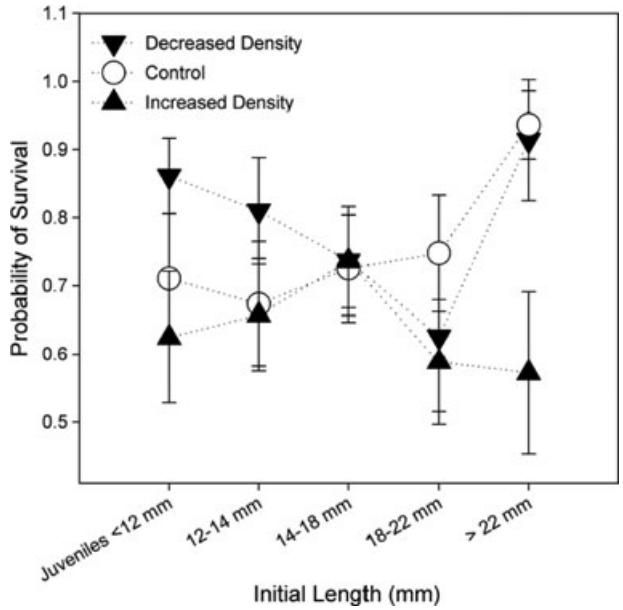

Figure 3. Least-square means from linear mixed model analysis of survival. Error bars are ± 1 standard error.

of guppies from low-predation environments normally experience density regulation. However, the demographic response to increased population density was different from the response to decreased population density.

When population density was increased, there was an increase in adult mortality rates, a decrease in individual growth rates, a reduction in fat reserves in adult females, and a reduction in offspring size. There was no change in fecundity. The latter three results suggest that there would be a reduction in fecundity in the longer term. Females were using fat reserves to sustain fecundity, but cannot do so indefinitely. Furthermore, reduced individual growth rates are associated with delayed maturity, maturity at a smaller size and reduced fecundity (Reznick 1982; Reznick and Endler 1982).

When population density was reduced, we instead saw a trend toward higher fecundity, increased individual growth rates, and increased juvenile survival rates, but no change in fat reserves. The increased individual growth rates will likely project to greater increases in fecundity in the future because increased growth is associated with earlier maturity, maturity at a larger size, and higher fecundity independent of size (Reznick 1982; Reznick and Endler 1982).

This difference in the responses to increased versus decreased population density yields some clues about how guppies may have evolved in response to fluctuating resource availability. They respond to a short-term decline in resources by drawing down fat reserves to sustain fecundity. They respond to a short-term increase in resource availability by increasing growth rate and RA, but not fat reserves. Both responses appear to put the priority on sustaining reproduction at the expense of storage and maintenance. It is thus possible that the manifestation of a uniform priority in response to either an increase or decrease in resource availability is the ultimate cause of the asymmetry in the demographic responses to changes in density.

When life-history theory has dealt explicitly with the demographic mechanisms that underlie population regulation, it has primarily modeled the consequences of single changes, such as a decline in fecundity alone or an increase in juvenile mortality rate alone (Charlesworth 1994). The complexity of the demographic response to density raises the question of whether there is any way to link it with existing theory. Caswell (1989) presents a potential bridge between empiricism and theory with his proposal that data such as ours can be viewed as a "life table response experiment." Our density manipulations constitute such an experiment because we can reconstruct a life table for each of the treatments, generate an estimate of population growth rate for each of them, then quantify the contribution of each demographic element to the differences in population growth rates. These estimates are a measure of the relative contribution of each element to density regulation.

Oli et al. (2001) took this approach in their analysis of density manipulations of natural populations of Uinta ground squirrels. Their results, like ours, revealed that the response to density was a complex combination of changes in age-specific survival and recruitment. In their case, the lion's share of the change in population growth rate was attributable to the number of offspring weaned, so the apparent complexity resolved to the happy ending that a single variable dominated the outcome. If this work were associated with a study of life-history evolution, then it would be possible to apply a model that assumed that density regulation was acting on juvenile survivorship. In contrast, Fowler et al.'s (2006) experimental increases and decreases in density of the

grass *Bouteloua rigidiseta* revealed a complementary decrease or increase in population growth rate, suggesting that natural populations were density regulated; however, no individual components of the life history stood out in governing the responses to the density manipulations. In other studies in which one or a very few factors dominated the demographic response, there was no consistency in which demographic response played the dominant role in governing density regulation (e.g., Gustafsson and Ehrlen 2003; Macdonald et al. 2009). In these latter studies, an associated model of life-history evolution would also have to incorporate the effects of density on reproduction and the differential impact of density on different age classes.

GUPPY LIFE-HISTORY EVOLUTION

Our empirical estimates of size-specific mortality revealed a mismatch between the mortality rates in high- versus low-predation environments and the life-history theory we had used to predict the course of guppy life-history evolution in response to predation (Reznick et al. 1996). The nature of the mismatch suggested the potential importance of density regulation in shaping guppy life-history evolution. Here we show that guppies from low-predation environments are indeed density regulated.

One way to consider how density regulation could come into play in the evolution of the low-predation life history is to envision the scenario of guppies from a high-predation locality invading, then adapting to a previously guppy-free locality that contained only *Rivulus*. We polarize the scenario as movement from down- to upstream because it follows our introduction experiments (Reznick et al. 1990, 1997), but is also consistent with the patterns of genetic variation that we see in natural populations, because genetic diversity is highest in the high-predation localities, which tend to be higher order streams, then declines in the lower order streams (Carvalho et al. 1996).

Invaders will initially be present at low population densities, then population density will increase as a consequence of the release from predation and hence reduced risk of mortality. In a new series of introduction experiments, with fourfold replication, we found that densities exceeded those typical of low-predation environments within a year of the introduction (unpubl. data). In associated experiments (Bassar et al. 2010b), we have shown that such an increase in guppy population density causes a significant reduction in resource availability, quantified either as algal standing crop or invertebrate abundance. We have also shown that guppies from low-predation environments are less specialized than those from high-predation environments in their diet; they feed unselectively on invertebrates, algae and detritus, while guppies from high-predation environments feed selectively on invertebrates (Bassar et al. 2010b; Zandona et al. 2011). Together, these studies suggest that the kind of selection guppies experience in low-predation environments is a combination of the reduction in mortality risk, then increased population density, followed by their consequent impact on the environment, which included depleting food resources.

The results reported here show that the near-term consequences of increasing population density on guppy demography will include a decline in fat reserves in adult females, reduced growth rates, reduced fecundity, and increased mortality. The increase in mortality is not equally distributed across all age/size classes. It is greater for the smallest and largest size classes (Fig. 3), with the largest size classes (adult females only) experiencing the largest effect. There is little or no change in mortality rate in the middle size classes, which corresponds with the size at maturity in males and females (Fig. 3) (Reznick and Bryga 1996). Adult fish are much more likely to emigrate (Reznick et al. 1996). Because we cannot discriminate between mortality and emigration in the current study, we cannot infer from these results alone whether the largest size class sustains the greatest density-dependent increase in mortality risk. The observed reduction in survival of the smallest size class is likely to be exclusively mortality because this size class rarely emigrates (Reznick et al. 1996).

OFFSPRING SIZE

Reznick and Yang (1993) performed an experiment in which isolated, adult female guppies were maintained on either high or low food rations. Females responded to low food rations by producing larger offspring. This plasticity appeared adaptive because large offspring have higher fitness than small offspring when food availability is low, but not when food is abundant (Bashey 2006). This effect of enhanced size at low food availabilities should be especially strong when there is competition among juveniles for resources (Brockelman 1975; Rodd et al. 1997). Here we found that increased density caused a reduction in offspring size while at the same time causing other phenotypic changes that we normally associate with reduced food availability (e.g., reduced growth rate, reduced fat storage in females). The reduction in offspring size thus appears to be a signature of a proximate effect of density that is independent of food availability and, by our earlier logic, is a maladaptive response to the environmental circumstances that accompany the density manipulation. It also does not correspond to how the guppy life history is destined to evolve, because guppies evolve to produce larger offspring in these low-predation environments (Reznick 1982; Reznick and Endler 1982; Reznick et al. 1990). Rodd et al. (1997) did not find an effect of density on offspring size in a laboratory study where guppies were reared in groups and food was maintained at approximately equal amounts per capita. In contrast, Leips et al. (Leips et al. 2009) found that the least killifish, *H. formosa*, another member of the poeciliid

family, responded to the experimental manipulation of density in aquaria by producing significantly larger offspring.

SENESCENCE

There is a third result from our previous research on aging in guppies that does not conform well with some theories of life-history evolution. Medawar (1952) and Williams (1957) predicted that the adaptation of guppies to the high-predation environment should be accompanied by the evolution of a higher rate of senescence in comparison to guppies adapted to low-predation environments for two reasons. First, selection for earlier maturity and higher rates of investment in reproduction early in life should be associated with costs that result in reduced fitness at later ages (Williams 1957). Second, reduced life expectancy in high-predation environments should cause reduced selection to sustain fitness in older age classes (Medawar 1952). When we compared the patterns of senescence in high- versus low-predation guppies in the laboratory, we found that the opposite was true; guppies from high-predation environments have lower intrinsic mortality rates, longer life spans, and higher fecundity throughout their lives than guppies from low-predation environments (Reznick et al. 2004).

At face value, these results suggest that guppies adapted to high-predation environment are "super" guppies and that the low-predation life history should never evolve, yet it does. We can exclude genetic drift as a likely explanation, because the level of genetic variation associated with one of our introduction experiments shows that there was not a genetic bottleneck associated with the establishment of the introduced population (Carvalho et al. 1996), yet the early life history evolved to be typical of low-predation environments.

Our finding that high-predation guppies are "super" guppies is potentially explained if guppies adapted to low-predation environments prove to have higher fitness in high population density. Population density was not a factor in the senescence experiment because fish were reared one per aquarium on quantified rations. If high-predation guppies lose their fitness advantage when reared at high population densities, then such genotype-by-environment interactions for life span and late-life fitness suggests that late-life fitness evolves as a by-product of adaptation to either density (Charlesworth 1980; Abrams 1993) or condition-dependent risk of mortality (Abrams 1993; Williams and Day 2003).

To summarize, we have shown that guppies are density regulated and have characterized the demographic mechanisms that enable density regulation. The demographic responses to increased density are different from those to decreased density, but both may be attributable to the same evolutionary response to fluctuating resource availability. This response appears to be to sustain reproduction at the expense of fat storage. The complexity of the demographic response means that there is not a neat fit between our situation and existing life-history theory, because existing theory tends to consider only a few simple demographic mechanisms for density regulation. Nevertheless, we are a step closer to reconciling life-history evolution in nature with the theoretical modeling of the evolution of life histories.

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Supporting Information

The following supporting information is available for this article:

Table S1. *F*-values and numerator and denominator degrees of freedom from linear mixed models.
Table S2. Random effect and error variance estimates and *Z*-statistics from linear mixed effects models.

Supporting Information may be found in the online version of this article.

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