



Competition as a selective mechanism for larger offspring size in guppies

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Highly competitive environments are predicted to select for larger offspring. Guppies *Poecilia reticulata* from low-predation populations have evolved to make fewer, larger offspring than their counterparts from high-predation populations. As predation co-varies with the strength of competition in natural guppy populations, here I present two laboratory experiments that evaluate the role of competition in selecting for larger offspring size. In the first experiment, paired groups of large and small newborns from either a high- or a low-predation population were reared in mesocosms under a high- or a low-competition treatment. While large newborns retained their size advantage over small newborns in both treatments, newborn size increased growth only in the high-competition treatment. Moreover, the increase in growth with size was greater in guppies derived from the low-predation population. In the second experiment, pairs of large and small newborns were reared in a highly competitive environment until reproductive maturity. Small size at birth delayed maturation and the effect of birth size on male age of maturity was greater in the low-predation population. These results support the importance of competition as a selective mechanism in offspring size evolution.

That the size of offspring a female produces reflects a balance between offspring number and fitness is a well-established tenet of life-history theory (Smith and Fretwell 1974), which has been documented in several empirical studies (Sinervo et al. 1992, Carriere and Roff 1995, Einum and Fleming 2000). However, except for a few cases (Iguchi and Yamaguchi 1994, Fox et al. 1997), what factors lead to shifts in offspring size is not well understood. Low resource environments are thought to be one of the major selective factors on offspring size (Brockelman 1975, Sibly and Calow 1983, Parker and Begon 1986). In these highly competitive environments, small offspring are expected have much lower fitness than large offspring such that the benefit of making larger offspring is predicted to outweigh the cost of making fewer of them.

Increases in offspring size have been shown to be correlated with low growth environments in comparisons both across field populations (Berven 1982, Orton and Sibly 1990, Tamate and Maekawa 2000, Johnson and Leggett 2002, Gregersen et al. 2006) and in response to seasonal changes within populations (Brody and Lawlor 1984, Landa 1992). However, in none of these cases has the hypothesis that competition is the mechanism driving the shift in offspring size been evaluated. Whereas experimental studies on various systems, from plants (Winn and Miller 1995) to invertebrates (Tessier and Consolatti 1989, Marshall et al. 2006), fish (Hutchings 1991, Einum and Fleming 1999) and amphibians (Berven and Chadra 1988, Parichy and Kaplan 1992), have all shown that larger

offspring have higher fitness in more competitive environments. Nevertheless, this pattern is not universal (Kaplan 1985, Ruohomaki et al. 1993, Svensson and Sinervo 2000). Therefore in order to demonstrate the importance of competition in the evolution of offspring size, what is needed are studies that pair a field pattern of association between the competitive environment and offspring size variation with empirical tests of the role of competition.

The guppy system provides the opportunity to make this connection. In guppies *Poecilia reticulata*, genetic differences in offspring size are correlated with habitat variation. Guppies from upstream, low-predation sites have offspring that are approximately 50% larger in dry weight than offspring from downstream, high-predation sites (Reznick and Endler 1982, Reznick and Bryga 1987, Reznick et al. 1996). This difference is heritable (Reznick 1982, Reznick and Bryga 1996) and has been shown to evolve in response to a change in predation regime in a field-based introduction experiment (Reznick et al. 1990). In addition, the competitive environment has been shown to vary with predation regime (Reznick et al. 2001). Populations from low-predation locales have four times the guppy biomass per unit area as populations in high-predation locales; yet, the primary productivity at low-predation sites is lower. Consequently, the growth rates of juvenile guppies are lower in low-predation locales (Reznick et al. 2001). Furthermore, field mark-recapture on small juvenile and newborn guppies has found no evidence that predation selects for the evolved differences in offspring size seen across predation

regime (Bashey 2002). Larger newborns were found to have a greater growth advantage in low-predation sites than in high-predation ones (Bashey 2002), suggesting that the competitive environment may select for offspring size differences.

Here, I present two laboratory-based tests of the hypothesis that variation in offspring size seen in guppies is an adaptive response to the competitive environment. I explicitly examined the effect of competition on the relationship between offspring size and offspring growth rate by rearing paired groups of large and small newborns in mesocosms created to mimic either a high- or low-competition guppy community. Additionally in a second experiment, I reared large and small newborn pairs in a highly competitive environment until sexual maturity in order to assess the duration of the fitness consequences of offspring size variation. Both experiments were conducted on guppies derived from a low- and a high-predation population and thus also allowed me to test whether populations differ in the consequences of offspring size variation.

Methods

Mesocosm competition experiment

I evaluated the consequences of offspring size on offspring growth as a function of population of origin and competitive environment. Guppies from one low-predation and one high-predation population were used. In each trial, I introduced a group of Small and a group of Large newborn guppies into a mesocosm that contained a background population of guppies at either Low or High density; both background fish and newborns were from the same source population. Each trial lasted for two weeks. A total of 24 trials were conducted over a four month period with 6 replicates for each population at High density and five and seven replicates for the low- and high-predation populations, respectively, at the Low density. The four treatments (two populations \times two competitive environments) were applied as evenly as possible such that there was no bias over the time course of the experiment.

Mesocosms consisted of 1000 l tanks designed to mimic Trinidadian stream pools. Each tank had a flowing “waterfall” and a shallow “stream edge”. The bottom of each tank consisted of “bedrock” region near the water inflow pipe and gravel elsewhere. In addition, two plastic plants, two yarn mops and two plastic tunnels were added to each tank to mimic the leaf litter, boulders, and tree branches found in Trinidadian stream pools. Mesocosms were housed in a greenhouse at UC Riverside and exposed to ambient, shaded light. Water quality (temperature = 23.5–24.5°C, pH = 7.2–7.4) was maintained with a flow-through heating/cooling/carbon filtration system. Each mesocosm had a surface area of approximately 1.79 m² and a volume of 0.282 m³. Six mesocosms were available for use and trials were assigned randomly to available mesocosms.

Both newborns and background fish were from laboratory stocks originally derived from two populations: high-predation, Oropouche (grid reference: QS 041 790) and low-predation, Quare “2/3 tributary” (PS 969 809). Stocks were collected from the field, subjected to controlled

breeding for two generations (Reznick et al. 2004), and then maintained in the laboratory in large, randomly breeding populations of approximately 1000 fish each. Approximately 60 F₂-F₄ females from each population were kept in group breeding tanks to generate newborns for this experiment. Newborns were collected over a 48 h period, standard length (SL) measured, and assigned to groups. Each group consisted of five newborns. Group association was determined such that the within group variation in SL was minimized while maximizing the difference between groups. Thus, the average size of newborns varied from trial to trial (mean, range: Small = 7.20 mm SL, 6.70–7.74; Large = 7.68 mm SL, 7.34–8.05), as did the initial difference between Large and Small newborns (0.48 mm SL, 0.1–1.16). Each group was marked at random by immersion in a 250 mg l⁻¹ solution of either calcein or alizarin red S for 24 h. These fluorescent dyes bind to calcium, are not visible under ambient light, and have proven to be an effective means for mark-recapture of small guppies (Leips et al. 2001, Bashey 2004).

Two competitive treatments were used: Low and High. Both competition treatments consisted of a background population of guppies with a size-structure that approximated a generalized guppy size-distribution (an average of the distributions found in low- and high-predation locales, Rodd and Reznick 1997). The Low treatment had 14 background guppies (1 6–8 mm SL, 2 8–10 mm SL, 2 10–12 mm SL, 1 12–14 mm SL, 1 14–16 mm SL, 1 16–18 mm SL, 1 18–20 mm SL, 2 20+ mm SL, and 3 mature males) and the High had 56 (4 \times for each size class). These two treatments gave densities approximating an average field density and an unusually high field density (Reznick et al. 2001). Both Low and High treatments were fed the same amount of food (0.2 ml of liverpaste/day or approximately 1594 J day⁻¹). Background fish were reared in the laboratory, measured and added to the mesocosms at the start of each experimental trial. To focus on early growth and facilitate comparisons with field mark-recapture studies (Reznick et al. 2001), trials were conducted for two weeks. At the end of a trial, all fish were collected from the mesocosm and SL measured. All juveniles (i.e. guppies < 12 mm SL) were checked for skeletal marks using portable fluorescence detectors (Leips et al. 2001, Bashey 2004). Only 3 of 240 focal newborn were not recovered; they were all Small newborns.

Statistical analysis

Because High and Low density treatments had significant heterogeneity of variances, separate analyses were performed for each density. I examined whether Large newborns retained their size advantage over Small newborns at the end of the experiment by examining the difference in mean SL at recapture for each newborn group paired by trial. I used ANCOVA (Proc GLM, SAS ver. 8.2) to see whether this difference was affected by population, average initial size of the newborns, initial difference between Large and Small newborns, and the growth of the background fish. Guppy growth (SL_{final} - SL_{initial}), declines linearly with SL for guppies greater than 12 mm (Reznick et al. 2001), but can increase with SL for smaller individuals (Bashey unpubl.). Thus, the growth of the background fish was modeled for each trial as a

decreasing linear or concave-down quadratic function of SL. The predicted change in SL of a 9 mm juvenile fish from the better fitting model was used as an indicator of the juvenile growth environment.

Newborn growth was calculated for each size class in each trial as $(\ln(\text{mean SL}_{\text{final}}/\text{mean SL}_{\text{initial}}))$. In each case, newborn growth was analyzed using an ANCOVA with population and newborn size-class as fixed effects and the initial SL, the initial difference in SL, and the growth of the background fish in the experiment as covariates. Also, due to the paired nature of Large and Small newborns in each mesocosm, trial nested within population was used as a random effect.

One-on-one competition experiment

The goal of this experiment was to test whether variation in offspring size could affect characteristics of reproductive maturity, and if so, whether these effects differed between populations. Fish used in this experiment were from laboratory stocks originally derived from two populations: a high-predation, Yarra River (grid reference: PS 802 904) and a low-predation, Yarra Tributary (PS 802 913). Stocks were collected and maintained as in experiment 1. Newborns were collected over a 24 h period from F₁–F₃ females held in group breeding tanks. Newborns were anesthetized and SL was measured to the nearest 0.01 mm. Two newborns from the same population (1 Large vs 1 Small) were put into an 8 l tank with one larger fish as a competitive background (average newborn size = 7.06 ± 0.04 mm SL, average difference between newborns = 0.50 ± 0.04). Seventy trials (high-predation $n = 34$, low-predation $n = 36$) were started over a six week period at random with respect to population. Mature males from the same stock population were used as the background fish because they remain relatively constant in size after maturity, thus allowing for the background effect to be constant over the course of the experiment. One of the two newborns was chosen at random to be marked with calcein. Fish were measured in SL and checked for marks biweekly. Each trial was run until the newborns reached sexual maturity or died.

Aquaria were kept in a temperature controlled room (water temperature was maintained between 23.5–25.5°C) and exposed to 12L:12D cycle. Water quality (pH = 7.2–7.4, hardness 160–180 ppm) was maintained by biweekly partial water changes. Fish were fed a measured quantity of liver paste in the morning and brine shrimp nauplii in the afternoon. The food was measured volumetrically to the nearest 0.25 μl with a Hamilton micropipette (Reznick 1982). Food levels increased biweekly for the first three months of the experiment to allow for the growth of the newborns and then was held constant. The tank food level started at 24.2 J day^{-1} and was capped at 121.0 J day^{-1} . This is a low food level and represents a highly resource-limited environment for three fish. Adult females show evidence of food-limitation on a diet of 60.5 J day^{-1} (Reznick and Yang 1993). Uneaten food was never observed in tanks.

Statistical analysis

To test whether Large newborns retained their size-advantage over Small newborns and to examine the influence of different factors on this advantage, I performed a repeated-measures ANCOVA on the SL difference between Large and Small newborns over the first two months of age (Proc GLM). Population was used as a main effect and start date of trial, initial difference between newborns, and average size of newborns as covariates. There were no significant interactions between population and the covariates, nor heterogeneity of variances across populations.

Analyses of characteristics of maturity and first reproduction were complicated because it is not possible to determine the sex of guppies at birth; therefore, four types of tanks resulted: single-sex tanks (either both male or both female) or mixed-sex tanks (either the Small newborn is female and the Large male or vice versa). For each sex, I analyzed single-sex and mixed-sex tanks in different ways. In all analyses, start date of trial was assessed as a possible explanatory variable both graphically and by inclusion as a covariate; it was never significant, and for simplicity, analyses without start date are presented.

For the single-sex male tanks, I compared age and SL at maturity (assessed via anal fin development, Reznick 1990) of the Small newborn to the Large newborn using a paired t-test, thus each tank acted as its own control. I then used one-way ANOVAs to look for differences between populations in (1) tank-average age and SL at maturity and in (2) the difference between the Large and Small fish in a tank. For the males in mixed-sex tanks, I compared age and SL at maturity using a two factor ANOVA with the newborn size-class as one fixed factor (either Small or Large) and population as another. Male age and SL at maturity met the assumptions of these analyses. For analysis of females in mixed-sex tanks, I used a two-factor MANOVA to test for differences in age, SL, number of offspring, and average size of offspring at first reproduction as result of newborn size-class or population of origin. Number of offspring was not normally distributed and exhibited heterogeneous variance across populations so was excluded from the analysis. For single-sex female tanks, it was often difficult to determine objectively which female produced a litter, so the trials were ended when the first litter was produced. Both females were then dissected to check their maturity status. This procedure made it impossible to compare characteristics of first reproduction, so I tested whether Large newborns were more likely to reproduce before Small newborns by comparing the observed number of cases where this occurred to the expectations from a random binomial distribution (Sokal and Rohlf 1995).

Results

Mesocosm experiment

After two weeks in the mesocosms, Large newborns retained, and slightly increased, their size advantage over Small newborns (Fig. 1). Neither the growth of the background fish in the mesocosm, nor the population of

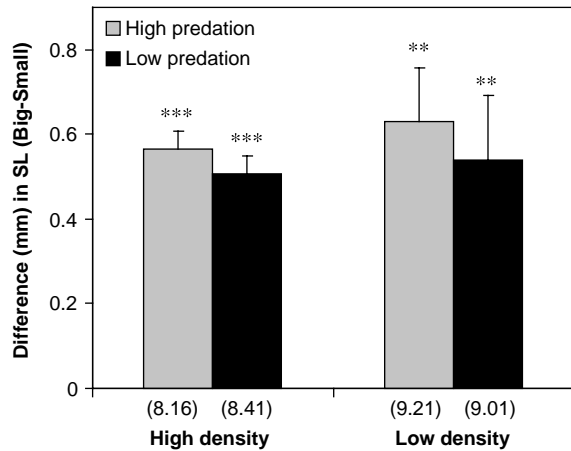


Fig. 1. Least-squares mean (± 1 SE) difference (Large – Small) in standard length (mm) between newborns in a mesocosm upon recapture for each source population and density treatment. Symbols centered above the bars indicate whether the difference between Large and Small newborns is significantly greater than zero (**0.001 < p < 0.01, ***p < 0.0001). Averages SL upon recapture for newborns in each treatment are given below the x-axis.

origin had a significant effect on the size advantage of Large newborns (Table 1). In the High density treatment, the initial difference between Large and Small newborns positively affected the final difference, while the average size of the newborns negatively affected it. At Low density, neither of these factors had a significant effect (Table 1).

Analyses of newborn growth showed a similar difference between density treatments in the effect of covariates. Additionally, they show that populations and newborn size classes differ in these effects. In the Low density treatment, the only factors that significantly influenced newborn growth were the growth of background fish and an interaction between population and the growth of background fish (Table 2, Fig. 2f). In mesocosms where the background fish grew better, newborns grew better. Furthermore, high-predation newborns grew significantly more than low-predation newborns in trials where the growth of the background fish was above average for the Low density treatments.

In the High density treatment, newborn growth was significantly influenced by background growth, initial difference and initial SL (Table 2). In this highly competitive environment, as the background fish grew better, newborns grew worse (Fig. 2e). Additionally, Small newborns were disproportionately affected by the initial

Table 1. Analyses of covariance on the difference in SL at recapture between the Large and Small newborns in a mesocosm for each density treatment. DF are 1, 11. None of the interactions between population and the covariates were significant.

Effect	High density		Low density	
	F	p	F	p
population	0.83	0.3921	0.20	0.6709
average initial SL	10.22	0.0151	0.02	0.8937
initial difference	49.25	0.0002	1.51	0.2582
background growth	0.06	0.8098	0.16	0.7055

difference in SL between the newborns as a larger gap between their size and that of their Large competitors resulted in better growth (Fig. 2g). In contrast, the growth of Large newborns was unaffected by the initial difference in SL between size classes (Fig. 2h). Moreover, the growth of Small newborns from the low-predation population was more positively affected by initial difference than their high-predation counterparts (Fig. 2g). Initial SL also positively affected the growth of both size classes (Fig. 2a-b). The strength of this effect was dependent on newborn size class as the growth of Small newborns was more positively affected by their initial size than was the growth of Large newborns (Table 2, see initial SL \times size). Moreover, initial size had a greater influence on growth of the newborns from the low-predation population than from the high-predation population (Table 2, see initial SL \times population). In fact, while growth rate increased significantly with size for newborns from the low-predation site, it did not for newborns from the high-predation site.

To summarize the High density results, Small newborns grew worse than Large newborns when the Small newborns were smaller than average or close in size to Large newborns. Furthermore, low-predation newborns grew significantly less than high-predation newborns at small birth sizes, but significantly more than high-predation newborns at larger birth sizes and larger initial differences.

One-on-one competition experiment

Effect of birth size on juvenile mortality and growth:

Mortality occurred in 13 of the 70 trials; 10 of these were within the first month of life. Small newborns were more likely to die than Large newborns (9 vs 4 deaths); however, this is not significantly different from a binomial distribution where Large and Small newborns have an equal probability of dying ($p = 0.1334$, Sokal and Rohlf 1995).

Large newborns were able to retain their SL advantage over Small newborns throughout the juvenile period (Fig. 3a). Repeated-measures ANCOVA of this difference showed a significant effect of the initial difference, but not of the average size of the newborns or trial start date (Table 3). The slope of the relationship between initial difference and final difference averaged 1.62 ($+/- 0.39$ SE) over the juvenile period. Thus initial differences in length at birth were maintained and perhaps compounded as the fish grew. The low-predation population showed a larger effect of birth size (a greater difference between Large and Small newborns over the juvenile period) than the high-predation population. In addition, this effect was longer lasting in the low-predation population as indicated by a significant interaction between population and week of measurement (Fig. 3a, Table 3).

Effect of birth size on age and size at maturity:

Males. Large newborns matured significantly earlier than Small newborns (Fig. 3b). This result was consistent between the single-sex (paired t-test $t = -1.86$, $n = 14$, $p = 0.043$ one-way) and mixed-sex ($F_{1,26} = 22.02$, $p < 0.0001$) tanks. As expected (due to known genetic differences, as well as a difference in size of background male), the high-predation populations matured significantly earlier than the

Table 2. Results of fixed effects from ANCOVA's on newborn growth rate at High and Low density in the mesocosm experiment. Trial was considered as a random effect to account for the paired nature of Large and Small newborns (size class). Population and size class were main effects, while initial SL, background growth, and initial difference were used as covariates. Interactions without F statistics were not significant and were dropped from the model (Milliken and Johnson 2002). When significant interaction between a covariate and main effect is present, the F statistic for the main effect is only valid when the covariate equals zero. All analyses were conducted using Proc Mixed (SAS ver. 8.2).

Effect	High density			Low density		
	DF	F	p	DF	F	p
Population	1,7	42.04	0.0003	1,8	4.30	0.0719
Size class	1,5	22.56	0.0051	1,8	0.00	0.9599
Pop * size	1,5	1.31	0.3049	1,8	0.30	0.5978
Initial SL	1,5	117.39	0.0001	1,8	0.00	0.9893
Background growth	1,5	7.39	0.0418	1,8	27.04	0.0008
Initial difference	1,5	85.00	0.0003	1,8	0.42	0.5349
Initial SL × size	1,5	22.39	0.0052	–	–	–
Initial SL × pop	1,5	41.05	0.0014	–	–	–
Background × pop	–	–	–	1,8	6.69	0.0323
Initial dif × size	1,5	74.68	0.0003	–	–	–
Initial dif × pop	1,5	17.77	0.0084	–	–	–
Initial dif × pop × size	1,5	36.22	0.0018	–	–	–

low-predation population (single-sex $F_{1,12} = 47.72$, $p < 0.0001$; mixed-sex $F_{1,26} = 90.70$, $p < 0.0001$). Populations also differed in the degree to which birth size influenced age at maturity. This difference was not significant for the single-sex tanks ($F_{1,12} = 0.96$, $p = 0.3465$), but was for the mixed-sex tanks as shown by a significant population by birth size interaction ($F_{1,26} = 9.45$, $p = 0.0049$). In each case, small birth size delayed maturation more in the low-predation population than in the high-predation population.

Size at maturity did not differ consistently between Large and Small newborns in either the single-sex (paired t-test $t = 0.06$, $n = 14$, $p = 0.9533$) or the mixed-sex ($F_{1,26} = 1.19$, $p = 0.2850$) tanks. Males from the low-predation population were larger at maturity ($F_{1,12} = 15.90$, $p = 0.0018$; $F_{1,26} = 11.85$, $p = 0.0020$), but there was no interaction between population and birth size ($F_{1,12} = .03$, $p = 0.8662$; $F_{1,26} = 0.19$, $p = 0.6633$).

Females. In 10 of 12 single-sex tanks, the Large newborn retained her size advantage over the Small newborn at the time of litter production; this is significantly different from the expectations of a random binomial ($p = 0.0193$, Sokal and Rohlf 1995). In all likelihood, the currently larger female was the mother of the litter; however, using objective criterion it was only possible to tell the mother of the litter in 8 of the 12 cases. For these, 7 had been Large newborns; again, this is significantly different from the expectations of a random binomial distribution ($p = 0.035$). It thus appears that Large newborn females are like Large newborn males in attaining sexual maturity at an earlier age than Small newborns.

For mixed tanks, there was no overall effect of birth size on characteristics of first reproduction (Wilks' lambda = 0.97, $F_{3,20} = 0.23$, $p = 0.8720$). Neither was there an interaction between birth size and population (Wilks' lambda = 0.79, $F_{3,20} = 1.77$, $p = 0.1848$). As expected, there was a significant effect of population on first reproduction, with high-predation females maturing significantly earlier and having smaller offspring (Wilks' lambda = 0.16, $F_{4,22} = 35.82$, $p < 0.0001$).

Discussion

The fitness consequence of a given reproductive bout depends not only on the number of offspring released into the environment, but also on their success (Smith and Fretwell 1974). In a benign environment, all offspring regardless of size may be successful. However, in a highly competitive environment, larger offspring may do disproportionately better such that a mother who makes fewer, larger offspring may have higher fitness than a mother who makes more, smaller offspring (Brockelman 1975). In this paper I report on two experiments that lend support to the hypothesis that larger offspring size increases offspring fitness in a competitive environment. In the first experiment, I show that offspring growth is dependent on offspring size in a high-, but not in a low-, competition environment. In the second experiment, I show that in a highly competitive environment, initial differences in size persist and affect maturation. Additionally, in both of these experiments, the consequences of offspring size variation are greater in guppies from the low-predation population. Below I discuss the findings of each experiment in more detail and conclude with the implications of this work.

Mesocosm competition experiment

The mesocosm experiment elucidates the effect of newborn size on the initial phase of guppy growth. Larger newborns were able to retain their size advantage over small newborns under both competitive treatments (Fig. 1). However, in the low-density treatment this advantage was more variable and not dependent on the initial size of or difference between newborns. In contrast in the high-density treatment, both of these factors were good predictors (Table 1). By further modeling newborn growth, it becomes evident that small differences in newborn size can influence growth rate and that this effect is environment specific. Newborn size significantly increased newborn growth only in the high-density treatment (Fig. 2a-b vs 2c-d). Moreover, the strength of this effect was greater for

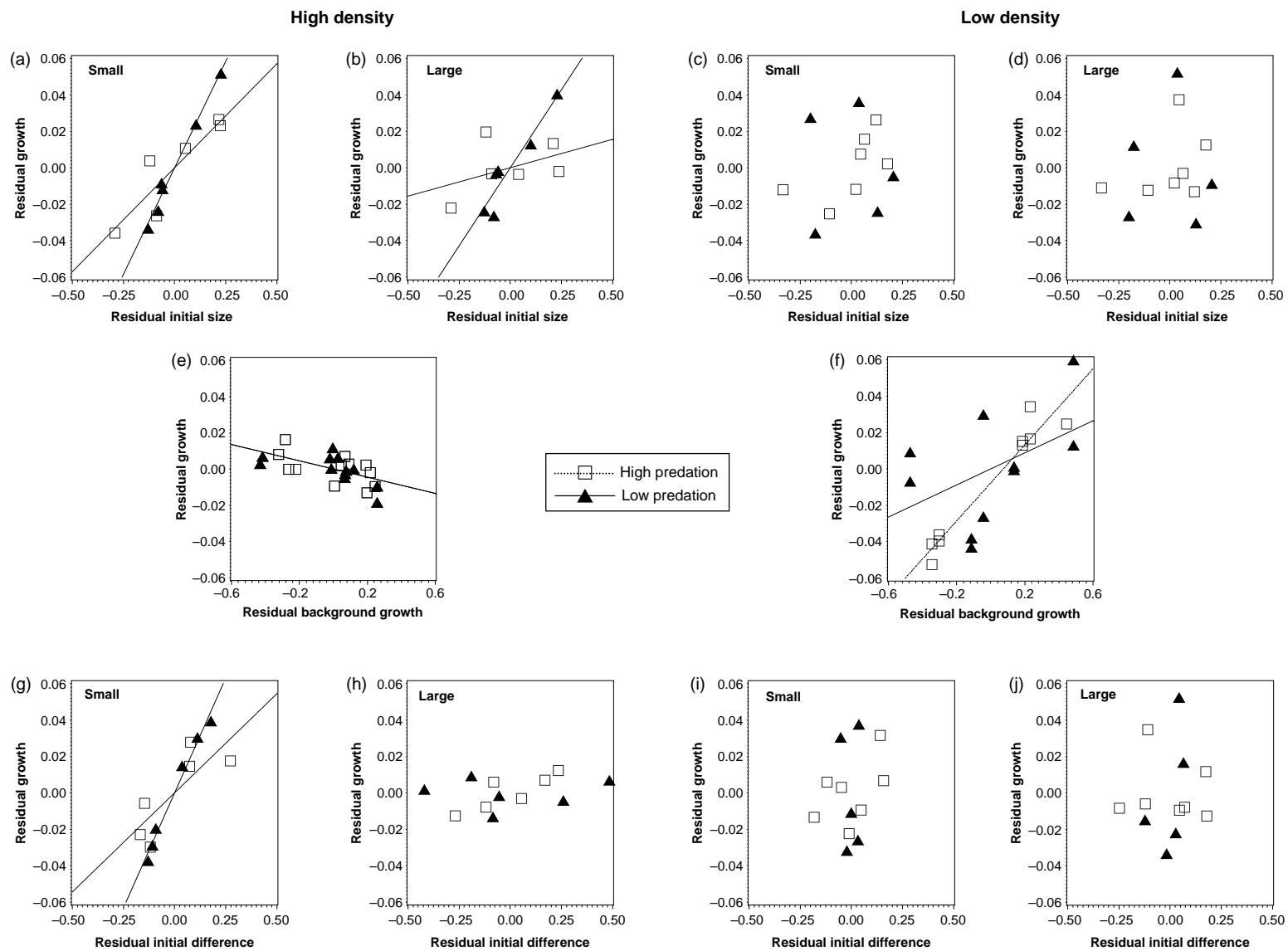


Fig. 2 (Continued)

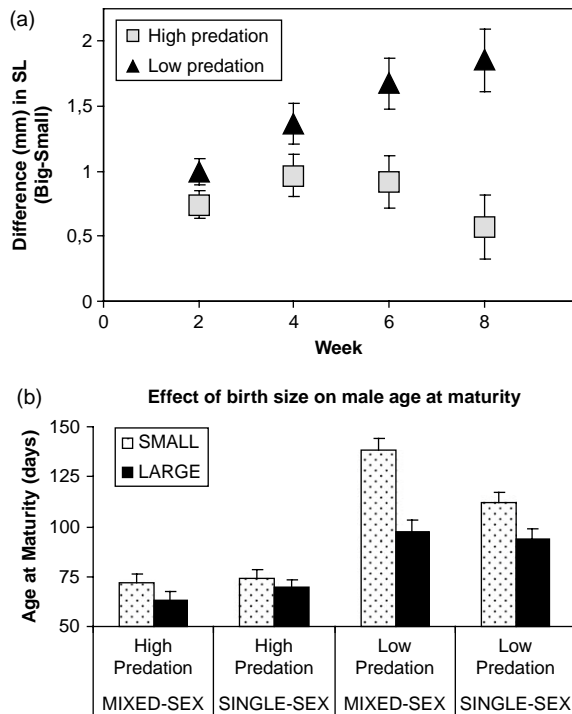


Fig. 3. (a) Least-square's mean (± 1 SE) difference in standard length between Large and Small newborns over the first eight weeks of life for each population. Large and Small newborns were paired in an aquarium in the presence of an adult male from the same population. Positive values indicate that Large newborns retained their size advantage over Small newborns. The effect of birth size was longer lasting in fish from the low-predation population. (b) Mean age at maturity (± 1 SE) of males classified as either Small or Large as newborns in the same experiment. Averages are presented for each population (Low predation vs High predation) and tank (mixed-sex or single-sex) combination. Mixed-sex tanks indicates that one newborn in the pair was male and one was female, while single-sex tanks indicate both were male. Note the greater effect of birth size on male maturity in the low-predation population.

small newborns than for large newborns. This difference between newborn size classes suggests diminishing returns on investment in offspring size via newborn growth (Lloyd 1987).

Further examination of the growth model also suggests that newborns in general (and small newborns in particular) are differentially affected by competition as assumed by many models of density regulation (Charlesworth 1994)

Table 3. Repeated-measures analysis of covariance on the difference in SL between Large and Small newborns in an aquarium. Each Large and Small newborn paired in an aquarium represents a subject in the analysis. None of the interactions between population and the covariates were significant. Sphericity was rejected, so multivariate tests for the within-subjects effects are given (Littell et al. 1996).

Effect	NDF	DDF	F	p
<u>Between subjects</u>	1	55		
population			5.68	0.0206
average initial SL			0.66	0.4191
initial difference			19.10	<0.0001
start date			0.54	0.4661
<u>Within subjects</u>	3	53		
week			0.32	0.8131
week \times population			4.87	0.0046
week \times initial SL			0.98	0.4082
week \times initial difference			1.44	0.2413
week \times start date			0.31	0.8196

and offspring size evolution (Brockelman 1975). For example, while the growth of the background fish in the experiment was positively correlated with newborn growth in the low-density treatment, it was negatively correlated in the high-density treatment (Fig. 2e vs 2f). This pattern suggests that in the resource rich, low-density treatment, background growth indicated the potential growth environment for newborns. In contrast, in the resource poor, high-density treatment, improved growth of the background fish indicated a more competitive environment for the newborns. Asymmetric competition between the newborn size classes was also implicated in the high-density treatment. The growth of small newborns was significantly affected by the size of the other newborns in their mesocosm, while the growth of large newborns was not (Fig. 2g vs 2h). For small newborns, a larger gap between their SL and that of their large competitors resulted in a higher growth rate suggesting that similarly sized newborns negatively influenced each other's growth, either through agonistic behaviors or by exploiting similar foraging strategies.

Source population also had a significant effect on newborn growth in the high-density treatment. Larger SL at birth and larger initial difference between size classes both had a greater influence on the growth of newborns from the low-predation population than from the high-predation population (Fig. 2a, 2b, 2g). Because I only competed newborns against a background of fish from their own populations, it is not possible to distinguish whether the

Fig. 2. Relationship between the growth of newborns in a mesocosm and (a-d) their average SL at birth, (e and f) the growth of background fish in the mesocosm, and (g-j) the initial difference in SL between Large and Small newborns shown at High and Low density. Each symbol represents the mean value of newborns in a given size class in a trial. Size class is indicated in the top left corner of the plot, except for e and f where both size classes are plotted on the same graph. Residuals are shown to control for the effect of the other factors and isolate the relationship between the variables plotted; this results in partial regression plots (Neter et al. 1990) and provides a close approximation of the slopes given by the models presented in Table 2. Here, only significant regression lines are shown. In (a) and (b), steeper slopes indicate a greater influence of newborn size on growth rate in the low-predation population and a greater influence of initial size on the growth rate of Small newborns. In (e) and (f), the effect of background growth does not differ between newborn size classes; however, the direction of the effect of background growth varies markedly with density and in the Low density (f) the high predation population responds more to background growth than the low predation population. In (g-j), Small newborns face decreased growth in a highly competitive environment if they are close in size to the Large newborns (g), but the growth of Large newborns is unaffected by the size of Small newborns (h), and neither size class is affected by initial difference in the low density treatment (i and j).

difference in slopes between populations is the result of different competitive levels imposed by the two background populations (i.e. low-predation fish provide a more intense competitive environment than high-predation fish), or whether traits conferring competitive ability have evolved (both independently of and correlated with offspring size) in low-predation newborns. Nevertheless under highly competitive conditions, newborns from the low-predation population faced a greater cost in terms of growth for being born small and a greater benefit of being born large than did those from high-predation population.

One-on-one competition experiment

Using two different populations, the laboratory experiment also showed a difference in the importance of offspring size congruent with differences in predation regime. In this experiment, the advantage of offspring size on growth was greater and longer lasting in the low-predation population (Fig. 3a). In guppies, increased juvenile growth can increase fitness by lowering the age and/or increasing the size of maturity (Bashey 2006, Reznick 1990). Moreover, the cues to initiate maturity vary with the growth environment (Reznick 1990). In the one-on-one experiment, males that were born small experienced delayed maturation and this effect was three-fold greater in fish from the low-predation population (Fig. 3b). It is again not possible to decouple differences in the competitive environment between the populations from evolved differences in their response to competition. Males from low-predation populations are larger at maturity than males from high-predation populations. Average male SL (± 1 SE) were 18.64 ± 0.18 mm for the low-predation population vs. 17.31 ± 0.17 mm for the high-predation, yet food levels were constant across populations potentially creating a more competitive environment for the low-predation replicates. Additionally, fish from the low-predation population are known to be more aggressive than those from high predation (Magurran and Seghers 1991). I also observed that low-predation males were more aggressive toward the newborns than those from the high-predation source. Nevertheless, the greater delay of male maturation for small newborns from low-predation guppies parallels the findings of Rodd et al. (1997) which demonstrated that maturation of male guppies from low-predation sites is more sensitive to density than that of their high-predation counterparts.

Females in the single-sex tanks showed a similar response as males: females that were larger as newborns reproduced sooner. Interestingly, this conclusion was not supported in the mixed-sex tanks. To reconcile the differences between the results from single- and mixed-sex tanks, I performed a power analysis for the effect of birth size on age at maturity. Given the observed variability in female age at first reproduction and my sample size, in order to detect a significant effect of birth size I would have needed an effect size (difference between age of first reproduction for large versus small newborns) of 10 days. In the single-sex tanks, I estimated the effect size to be approximately 15 days. Clearly the mixed-sex tanks did not exhibit the same degree of response as the single-sex tanks. This disparity could be due to different competitive abilities of males and females,

such that in mixed-sex tanks an initially small, competitively inferior female newborn would, in time, be able to out-compete her male counterpart. Alternatively, it could be due to the different social pressure faced by the different sexes in the experiment. Both male and female guppies have been shown to vary their reproductive traits in response to social environment, but female responses are more slow to manifest (Rodd et al. 1997, Rodd and Sokolowski 1995). It would be interesting to explore further whether there are differences between the sexes in the importance of birth size. Such sex differences could lead to differential investment within a clutch, although that may be unlikely in organisms where maternal investment is fixed before fertilization.

Implications for the evolution of offspring size

Guppies from low-predation sites have evolved larger offspring than guppies from high-predation sites (Reznick 1982, Reznick et al. 1990, Reznick and Bryga 1996). However, as numerous factors vary across these sites (Magurran 2005, Reznick et al. 2001), the selective pressures that have led to this difference may be complex. In this study I demonstrate that offspring size affects offspring growth only in a competitive environment, and that in a competitive environment, initial differences in offspring size can persist and affect the timing of first reproduction. Other studies also support the view that the competitive environment may have been a key mechanism in the evolution of guppy offspring size. For example, Grether et al. (2001) compared guppies from two low-predation streams in a common garden and found that guppies from the low-light (high-competition) stream produced larger offspring than offspring from the high-light stream. Additionally, guppies plastically increase the size of their offspring size in response to low maternal food (Reznick and Yang 1993), which results in a maternal effect on offspring fitness only in a low-resource environment (Bashey 2006). Finally, experimental manipulation of guppy density in the field had a greater effect on the survival and growth of newborns and juveniles than adult survival or fecundity, and this density effect only occurred at low-predation sites (Bronikowski et al. 2002).

In conclusion, this study supports the hypothesis that offspring size differentiation in guppies is an adaptation to the competitive environment. That other life-history traits may also be viewed as evolving in response to resource level rather than predators (Arendt and Reznick 2005), does not negate the importance of predation in guppy evolution. Rather, it stresses the importance of testing selective hypotheses and the need to determine the relative importance of different causal factors (Reznick et al. 2002).

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