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LIFE-HISTORY EVOLUTION IN GUPPIES (*POECILIA RETICULATA*)

6. DIFFERENTIAL MORTALITY AS A MECHANISM FOR NATURAL SELECTION

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Abstract.—We have previously reported a correlation between the life-history patterns of guppies and the types of predators with which they coexist. Guppies from localities with an abundance of large predators (high predation localities) mature at an earlier age and devote more resources to reproduction than those found in localities with only a single, small species of predator (low predation localities). We also found that when guppies were introduced from a high to low predation locality, the guppy life history evolved to resemble what was normally found in this low predation locality. The presumed mechanism of natural selection is differences among localities in age/size-specific mortality (the age/size-specific mortality hypothesis); in high predation localities we assumed that guppies experienced high adult mortality rates while in the low predation localities we assumed that guppies experienced high juvenile mortality rates. These assumptions were based on stomach content analyses of wild-caught predators and on laboratory experiments. Here, we evaluate these assumptions by directly estimating the mortality rates of guppies in natural populations. We found that guppies from high predation localities experience significantly higher mortality rates than their counterparts from low predation localities, but that these higher mortality rates are uniformly distributed across all size classes, rather than being concentrated in the larger size classes. This result appears to contradict the predictions of the age/size-specific predation hypothesis. However, we argue, using additional data on growth rates and the probabilities of survival to maturity in each type of locality, that the age-specific mortality hypothesis remains plausible. This is because the probability of survival to first reproduction is very similar in each type of locality, but the guppies from high predation localities have a much lower probability of survival per unit time after maturity. We also argue for the plausibility of two other mechanisms of natural selection. These results thus reveal mortality patterns that provide a potential cause of natural selection, but expand, rather than narrow, the number of possible mechanisms responsible for life-history evolution in guppies.

Key words.—Adaptation, life-history evolution, mark-recapture, mortality, selection, size-selective predation.

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Testing evolutionary theories in natural populations requires identifying the agent of selection, evaluating how the agent operates, then manipulating it and observing the resulting dynamics of evolutionary change (Endler 1986). Satisfying these criteria is rarely possible, so inferences are more often made less directly, such as with the combination of comparative studies and genetics that comprise the discipline of ecological genetics (e.g., Ford 1971). In initial studies (Reznick 1982; Reznick and Endler 1982), we found an association between predator fauna and the life-history attributes of their prey. We then demonstrated that a change in predator fauna could select for a change in the life history of the prey (Reznick and Bryga 1987; Reznick et al. 1990). Indirect evidence indicated that the mechanism selecting for these life-history patterns was predator mediated differences in age-specific survival. Here we evaluate this mechanism using direct estimates of survival rates in natural populations.

One theory for the evolution of life-history traits that provides a possible explanation for our results is the age-specific mortality hypothesis. It predicts that an increase in the mortality rates of adults relative to juveniles will select for individuals that attain maturity at an earlier age and have a higher rate of allocation of resources to reproduction. Conversely, a selective increase in juvenile mortality rates favors delayed maturity and reduced allocation to reproduction (Gadgil and Bossert 1970; Law 1979; Charlesworth 1980; Kozłowski and Wiegeert 1987). We evaluated this theory first

with comparative studies of a large number of natural populations of guppies (*Poecilia reticulata*) that coexist with different species of predators in streams in Trinidad. Our comparisons were primarily between guppies from *Rivulus* and *Crenicichla* localities. The killifish *Rivulus harti* is the main guppy predator in smaller streams and has been characterized as preying primarily on small, immature size classes of guppies. *Crenicichla alta*, a pike cichlid, and associated predators have been characterized as preying predominantly on large, reproductive size classes of guppies (Haskins et al. 1961; Liley and Seghers 1975; Seghers 1973, 1978; Endler 1978). We found that guppies have heritable life-history patterns consistent with the predictions of the age-specific mortality hypothesis (Reznick 1982, 1989; Reznick and Endler 1982; Strauss 1990); guppies from *Crenicichla* localities attain maturity at an earlier age and have higher rates of investment in reproduction than their counterparts from *Rivulus* localities.

We further evaluated this theory by manipulating the kind of predation that guppies were exposed to (Reznick and Bryga 1987; Reznick et al. 1990). We did so by introducing guppies above a barrier waterfall that had previously excluded all fish except *Rivulus*. Below the barrier, guppies were found in a typical *Crenicichla* community. This introduction presumably released guppies from selective predation on adult size classes (*Crenicichla* locality) and exposed them instead to selective predation on juveniles (*Rivulus* locality).

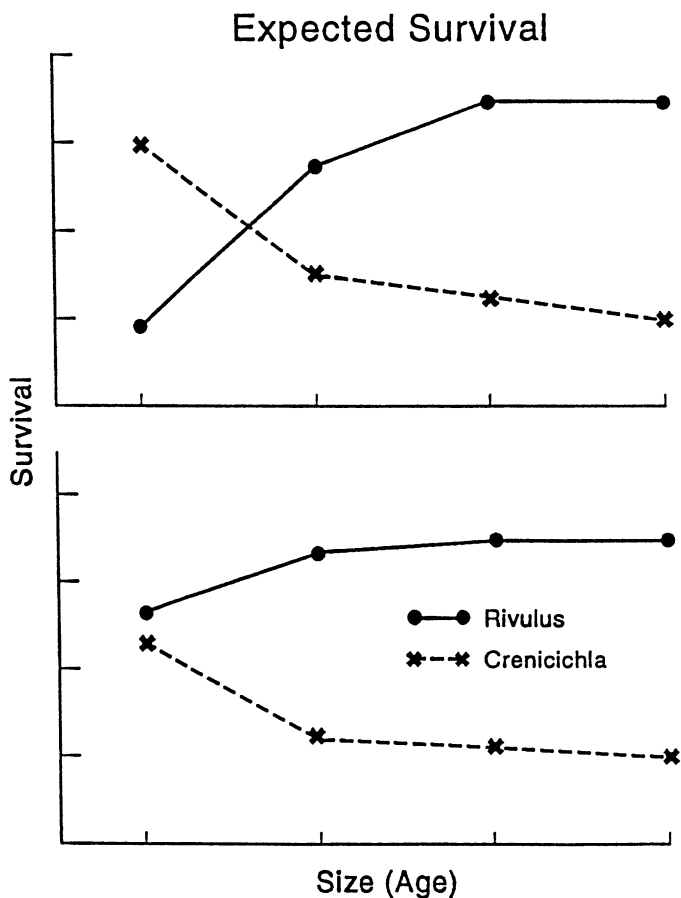


FIG. 1. Predicted recapture probabilities, if the age-specific predation hypothesis is true. The x-axis represents the size distribution of guppies. The y-axis represents the probability of survival during some time interval. See text for explanation of these predictions.

After 11 years, or 20–40 guppy generations, we found that guppies from the introduction site attained maturity at a significantly later age and allocated fewer resources to reproduction than controls from below the barrier waterfall, and that these differences were heritable (Reznick et al. 1990), again as predicted by the age-specific mortality hypothesis. All available evidence thus indicates that life-history theory accurately predicts life-history evolution in natural populations of guppies. What remained untested was the actual differences in mortality rates of guppies in *Rivulus* and *Crenicichla* localities and hence the mechanism of natural selection.

Here, we test the age-specific mortality hypothesis with mark-recapture experiments. If the age-specific predation hypothesis is true, then we predict that the mortality rates of juvenile guppies relative to adults will be high in *Rivulus* localities. We predict the opposite mortality pattern in *Crenicichla* localities (Fig. 1). Mortality rate is estimated as (the probability of recapture) after a fixed interval of time. There is some flexibility in the kind of results that will support this hypothesis (Fig. 1). In Figure 1A, the hypothetical differences in the mortality rates of juveniles versus adults cross over, so that guppies from *Crenicichla* localities suffer lower mortality rates as juveniles and higher mortality rates as adults

TABLE 1. Localities and approximate grid references for the mark-recapture studies. The number in parentheses represents the number of pools used for mark-recapture experiments.

A. High predation localities			
Ceniza River (3):	PS	960	797
El Cedro River (1):	PS	896	788
Mausica River (3):	PS	849	790
B. Low predation localities			
Aripo Tributary (3):	PS	931	799
El Cedro River (2):	PS	895	797
Quare Tributary (2):	PS	967	808

in comparison to *Rivulus* localities. In Figure 1B, the guppies from *Crenicichla* localities suffer higher mortality rates in all size classes, but the magnitude of the differences increase with the size of the prey. What the two hypothetical results have in common is the nature of the interaction between mortality rate, guppy body size, and predator community. It is this interaction, or the relative mortality rates of juveniles and adults in each type of community, that we seek to evaluate. In either case, we are evaluating the hypothesis that differences in the mortality rates of juveniles versus adults have led to the evolution of the distinct suites of life-history traits that we have observed in guppies.

MATERIALS AND METHODS

Study Sites

All of our work was done in Trinidad, in small streams with distinct riffle-pool structures. Guppies tend to congregate in pools and are rarely found in riffles, so we treated individual pools as sampling units. Stream structure is thus a critical component of the experimental design. We worked in three different streams from each type of predator community. The *Rivulus* streams were a tributary to the Quare River, a tributary to the Aripo River, and the El Cedro River. The *Crenicichla* streams were the Ceniza River, the Mausica River, and the El Cedro River (see Table 1 for grid references). The *Crenicichla* and *Rivulus* sites on the El Cedro River were on the same tributary, with the *Rivulus* sites being found above a barrier waterfall that serves as the upstream border for *Crenicichla* and associated species of fish. All streams were of similar size, as were the pools selected for study within each locality. All experiments were done during the dry season, which enhances the degree of separation between pools. Note that both types of predator communities can also be found in larger streams that have more open channels, rather than pools and riffles. Such streams were not included in this study because of the difficulty of obtaining good mark-recapture results.

Crenicichla streams contain a diversity of fish species in addition to guppies and *Crenicichla*. The ones chosen for study generally included *Astyanax bimaculatus*, *Hemibrycon dentatum*, *Hoplias malabaricus*, and *Aequidens pulcher* among the diurnal fauna. The nocturnal fauna would generally include *Rhambdia sebae*, *Gymnotus carapo*, and *Synbranchus marmoratus*. Only a subset of these species were found in the pools chosen for study and only the diurnal species would have been included in our visual censuses. The

sites on the Mausica River included *Astyanax* and *Hemibrycon* and those on the Ceniza River included *Astyanax*, *Hemibrycon*, and *Hoplias*. While all of these species were also seen on the El Cedro, none were seen in the study site during the course of our experiment. Many of these other species will also include guppies in their diet (Endler 1978). It is important to note that none of them specialize on guppies. *Crenicichla*, which is generally considered to be the most severe guppy predator (Endler 1978) also includes crustacea, aquatic insects, snails, and other species of fish in its diet. We have also occasionally seen *Synbranchus* in *Rivulus* localities, but did not see any in our study sites.

Mark-Recapture Experiments

We collected fish from individual pools using butterfly nets in a fashion that did not modify the habitat in any way. Because guppies are curious and are attracted to sediment raised by maneuvering the net, we were able to catch all of the guppies from a given pool, usually within an hour or two. Each pool was resampled repeatedly after an interval of a few minutes until no additional fish were caught.

We evaluated our success in completely removing all guppies from a pool by repeatedly visiting and recollecting a series of pools for up to two weeks after the initial collection. We enumerated the guppies caught during each visit. We predicted that, if we were successful in collecting almost all of the fish on the first visit and if there was little movement from one pool to the next over short time intervals, then we would catch few additional fish on subsequent visits. High capture rates on subsequent visits could indicate either a lack of success in our initial collection or a high rate of movement among pools.

We then conducted mark-recapture studies at a total of seven *Crenicichla* and seven *Rivulus* sites over a four year period (1988–1991) (Table 1). We included *Rivulus* and *Crenicichla* localities in each year of the study. For each of the 14 sites, we collected all of the guppies from a single pool. Guppies were collected and marked on day 1, maintained in water medicated to prevent fungal or bacterial infections, released on day 3, then recollected on day 14. When recollecting marked guppies, we censused pools upstream and downstream of the study pool to search for marked emigres. The small number of emigres that we found were included in the recapture data. We also recollected on subsequent days at some sites to see how many fish were missed during the first visit. We paint-marked and released a total of 1286 guppies > 12 mm standard length (SL) (all lengths are measured from the tip of the lower jaw to the posterior margin of the hypleural plate) and calcein marked and released 589 guppies < 12 mm SL.

We marked guppies > 12 mm SL by injecting a dot of acrylic latex paint in the caudal peduncle. The paint was diluted to a 30% solution with sterilized Teleost Ringers (5.5 g/l NaCl, 0.14 g/l KCl, 0.12 g/l CaCl₂) and injected with a 30 gauge needle. We marked 16 distinct size classes by using two colors (red and black) and eight positions on the caudal peduncle. Guppies 12–25 mm SL were marked according to their millimeter size class. Individuals that were 26–28 mm and those > 28 mm were marked as two distinct size classes.

These larger size classes were rare and only included adult females. We marked guppies < 12 mm by immersing them in a 250 mg/L solution of calcein 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 with an epifluorescence microscope (following Rodd and Reznick 1991). In laboratory studies, we established that our paint marks are retained for > 12 mo and that growth and survival are unaffected by marking. Paint and calcein-marked guppies held in the laboratory as controls during each field experiment confirmed mark retention for the duration of all field experiments.

We evaluated the influence of the marks on the probability of being eaten by *Crenicichla* or *Rivulus* following the methods of Mattingly and Butler (1994, p. 56–57) in their “small-tank tests.” Predators were maintained in a home aquarium of 8.8 l for *Rivulus* and 38–209 L for *Crenicichla*. The individual *Rivulus* ranged from 41–92 mm SL, while the *Crenicichla* ranged from 118–162 mm SL. Predators were starved for 3 d before an experiment. Six marked and six unmarked guppies, matched for size, were introduced into the test aquarium. After 24 h, the surviving guppies were removed and scored as being either marked or unmarked; missing guppies were assumed to have been eaten. The experiment was repeated using 10 individual *Rivulus* and nine individual *Crenicichla*.

Evaluating the Relationship between Age and Size

The age-specific mortality hypothesis compares the mortality rates of organisms of different ages. In all of our work, we have dealt instead with size-specific predation and mortality rates. It is thus necessary to evaluate the correlation between size and age. Such correlations are weak for many organisms, prompting the use of stage/size based, rather than age-based, life-history models (e.g., Werner and Caswell 1977). We evaluated the relationship between size and age in guppies by using otoliths, bonelike structures in the inner ear, to age wild-caught guppies. Many species of fish form daily rings on their otoliths (e.g., Taubert and Coble 1977); the daily periodicity of ring formation has also been demonstrated for guppies (D. Reznick, pers. obs.). We include a subset of our work on otoliths here to document the age-size correlation in guppies and hence determine if size is an acceptable surrogate for age.

We preserved wild-caught guppies in 95% ethanol. Otoliths were removed and placed in immersion oil for a week, which penetrates and clarifies the otoliths, making the rings more discernible. Ring counts were made on both lapilli, the middle sized otolith, by the same observer using a 40X plan-apochromatic lens. A subset of the individuals were scored by a second observer to confirm the repeatability of the counts. The values used for statistical analyses were the average of the readings on both otoliths. The data reported below are based on collections made from eight localities in February 1984. We extracted and scored otoliths from an average of 11 females per locality. The females were chosen to equally represent the following size classes: 12–13 mm, 17–18 mm, and 21–23 mm.

Statistical Analyses

Otolith Studies.—We evaluated the relationship between age and size (SL in mm) with analyses of covariance, with size as the covariate, locality as the independent variable, and ring number as the dependent variable. We evaluated the homogeneity of the slopes of the size–ring count regressions in a preliminary analysis and then, if this assumption was satisfied, repeated the analysis using a common slope. Our main goal in this analysis was to evaluate the relationship between estimated age and size.

Mark-Recapture Studies.—We analyzed the recapture data with a log-linear model (BMDP 4F; Dixon 1992). Individual fish were classified as coming either from a *Crenicichla* or *Rivulus* locality, and as having been either recaptured or not recaptured at the end of the experiment. The probability of recapture is assumed to be an index of the probability of surviving a given time interval. To enhance the power of the test, we reduced the number of size classes to four categories: (1) < 12 mm, this size class is entirely juveniles that are calcein marked; (2) 12–14 mm, these are almost exclusively juveniles; (3) 14–18 mm, these include females producing their first and possibly second litter (i.e., the size class with the highest reproductive value) and over 95% of the mature males; and (4) > 18 mm, these are sexually mature females that are producing second and later litters. These four categories were chosen a priori to provide sufficient size resolution and to be “stage specific,” meaning that sizes were combined to separate distinct phases in the life cycle. The size categories incorporate the same stages in both types of locality.

The “recapture \times predator” term in this model evaluates overall differences between *Rivulus* and *Crenicichla* localities in the probability of recapture. “Recapture \times size class” evaluates overall differences among size classes in the probability of recapture. “Recapture \times predator \times size class” evaluates differences among *Rivulus* and *Crenicichla* localities in the age/size-specific recapture probability, and hence mortality rate. This 3-way interaction incorporates the test of the age (size) specific predation hypothesis. Figure 1 illustrates the form that such interactions would take if there are the predicted differences among localities in age-specific survival. In alternative analyses, we used logistic regressions and nested localities within predators (Proc Catmod; SAS Institute 1985). These alternative analyses therefore explicitly incorporated differences among localities within a predator treatment. These alternatives yielded qualitatively similar results. For the sake of simplicity, we only report the results of the log-linear analyses.

RESULTS

Otolith Study: The Relationship between Size and Age

A preliminary analysis of covariance revealed that there was no slope heterogeneity among the ring-count, standard-length regressions for the eight localities ($F_{7,72} = 0.59$, $P = 0.7611$). In the subsequent analysis that assumed homogeneous slopes, we found that ring count was positively correlated with standard length ($F_{1,79} = 362.31$, $P < 0.0001$) and accounted for 72% of the total variation. There was also

significant variation among localities in length-adjusted ring count ($F_{7,79} = 3.16$, $P = 0.0054$), which implied that there were differences among localities in growth rate. Fish of a given length differ in ring count, or age, among localities implying that it took different intervals of time to reach that size. This variation among localities accounted for an additional 12% of the total variation. The association between ring count and size demonstrates that length is strongly, positively correlated with age. Size-specific predation can thus be assumed to influence age-specific survival.

Preliminary Investigations of Marking and Population Sampling

The Influence of a Paint Mark on the Susceptibility to Predation.—We tested whether paint-marked guppies might experience differential predation when compared with unmarked guppies. These tests revealed that marked and unmarked guppies were eaten with equal frequency, and that size selectivity of the predators was unaffected by marking (3-way test of independence; $G = 0.962$, $df = 1$, $P > 0.05$). The number eaten/number offered for *Rivulus* were 21/60 for marked and 20/60 for unmarked guppies. For *Crenicichla*, they were 18/54 for marked and 20/54 for unmarked guppies. We conclude that marks did not affect the short-term probability of predation on guppies.

Estimates of Collection Efficiency.—In repeated censuses of three pools, each in a different stream, the number of fish collected during the subsequent two week period was less than 5% of the number collected on the first visit (number caught on subsequent visits/number caught on first visit = 6/111, 8/163, 6/219). This suggests that we caught the majority of the residents on our first visit and that there is little short-term movement among pools. We then performed four small scale, mark-recapture studies using adult size-classes of females in *Rivulus* localities. Our expectation was that the recapture probability should be close to 100% because guppies of this size exceed the gape dimensions of most *Rivulus*. Twelve days after the release, we recaptured an average of 91% of marked fish in the same pool in which they were released (number recaptured/number marked and released = 34/37, 12/12, 12/13, 13/16). Based on these two types of studies, we concluded that it is possible to collect virtually all fish within a pool on the first visit and that recapture rates can serve as an index of survival.

Four examples of more complete evaluations of our success at recapturing marked guppies and their frequency of movement among pools (Table 2) again illustrate that recapture probability is a good index of survival. Three of these sites (Table 2A) were on the El Cedro River and were included in the comparisons among predator communities in mortality rate. Twenty of 398 recaptured, paint-marked individuals, or 5.03%, were recaptured outside of the release pool, again implying a low rate of emigration over short time intervals. We did not find any calcein-marked juveniles outside of the mark-release pools. No additional marked fish were caught in the two pools that were recollected on the second day, implying that the probability of recapture of marked fish on the first day was very high.

A 1993 experiment, not included in subsequent analyses

TABLE 2A. The location and timing of recapture of marked individuals in four studies: three mark-recapture experiments from the El Cedro River. ECD was a downstream, *Crenicichla* locality. ECU1 and ECU2 were two upstream, *Rivulus* localities. MR = the mark-recapture pool. U1 and U2 = the first and second pools upstream from the MR pool. D1 and D2 = the first and second pools downstream from the MR pool. The number in each cell represents the number of paint-marked fish (initial size > 12 mm) recollected in each pool 12 d after release. We revisited the ECD and ECU1 sites 13 and 14 d, respectively, after the release, but caught no additional marked fish. The total number of marked fish in each pool is indicated at the bottom of the columns for day 12, while the fraction recaptured is indicated in parentheses. ECD is the downstream control (*Crenicichla* locality) and ECU1 the introduction site (*Rivulus* locality). ECU2 is a second *Rivulus* locality further upstream. Probability of recapture is given in parentheses.

	ECD		ECU1		ECU2
	Day 12	Day 13	Day 12	Day 14	Day 12
Number recaptured					
U2	—	—	0	0	0
U1	0	0	12	0	0
MR	102	0	202	0	74
D1	5	0	2	0	0
D2	1	0	0	0	0
Number released	144 (0.75)		232 (0.93)		83 (0.89)

because it involved a different type of predator community, again evaluated the probability of recapture on the first visit and the probability of emigration. In this case, we had two mark-recapture pools separated by riffles and one pool. Ninety-two percent (58/63) of the paint-marked fish (> 12 mm) were recaptured, all on the first day and all in the pool in which they were released. Seventy-nine percent (33/42) of the calcein-marked fish were recaptured, all but one on the first day and all in the pools in which they were released. If we consider just the total number > 12 mm, both marked and unmarked individuals, caught on day 1 versus subsequent days, then the implication is that there was a > 99% probability (140/141) of being caught on the first day. For fish < 12mm

TABLE 2B. In this study, we had two mark-release (MR) pools separated by riffles and one intermediate pool (I). We collected in the two pools above the upper MR pool (U1 and U2) and the two pools below the lower MR pool (D1 and D2). We collected in all seven pools 12, 13, and 14 d after the release. The number of recaptured, marked fish are indicated outside of parentheses in each cell. The number of unmarked fish are indicated inside the parentheses. Fish < 12 mm were marked with calcein. Those > 12 mm were paint marked. The large number of unmarked fish < 12 mm in the MR pools primarily represent young born during the two week study period.

	Day 12		Day 13		Day 14	
	< 12 mm	> 12 mm	< 12 mm	> 12 mm	< 12 mm	> 12 mm
U2	0 (43)	0 (18)	0 (0)	0 (0)	0 (0)	0 (0)
U1	0 (0)	0 (3)	0 (0)	0 (0)	0 (0)	0 (0)
MR1	16 (17)	23 (1)	0 (1)	0 (0)	0 (0)	0 (0)
I	0 (0)	0 (4)	0 (0)	0 (0)	0 (0)	0 (0)
MR2	16 (11)	35 (1)	1 (2)	0 (0)	0 (0)	0 (0)
D1	0 (43)	0 (37)	0 (5)	0 (1)	0 (1)	0 (0)
D2	0 (15)	0 (18)	0 (1)	0 (0)	0 (0)	0 (0)
Total	32 (129)	58 (82)	1 (9)	0 (1)	0 (1)	0 (0)

TABLE 3A. Analysis of mark-recapture experiment results, which excludes mature males from the 14–18 mm size class. Reported here are the key chi-square tests, computed from the difference between the likelihood ratio chi-square for models with and without the interaction in question.

Effect	df	χ^2	P-value
Recapture \times predator	1	46.15	<0.001
Recapture \times size class	3	48.73	<0.001
Recapture \times predator \times size class	3	3.41	0.33

(juveniles too small to have received paint-marks) there was a 94% (160/171) probability of capture on the first visit, and > 99% probability (170/171) of capture on the second visit.

We conclude that the probabilities of a fish being alive but not captured on the first visit, or being alive but having emigrated out of the study site are quite low. Recapture probability is thus a good estimate of survival.

Comparative Mortality Rates

We first analyzed recapture probabilities for just females and immature males. We excluded mature males because they are brightly colored, while the other fish are a uniform, tan color. Mature males also perform conspicuous mating displays. These differences between mature males versus immature males and females are likely to influence predation rates (Farr 1975; Endler 1978). The recapture probability of all size classes, excluding mature males, was significantly lower at *Crenicichla* localities than *Rivulus* localities (Table 3A, Fig. 2A), indicating a higher mortality rate in *Crenicichla* localities. There were also significant differences among size classes in recapture probability. The recapture (survival) rates tended to increase with size, although the 14–18 mm size class had the highest recapture probability.

The 14–18 mm size class also includes mature males. To estimate the overall mortality rate of the 14–18 mm size class in comparison with smaller and larger fish, we repeated the analysis, including mature males with the 14–18 mm females and immature males (Table 2, 3B, Fig. 2B). The shape of the recapture profile changed, so that the significant influence of size is now attributable solely to the low survival of guppies < 12 mm SL. When the analysis was repeated without this smallest size class, there was no longer any significant size effect. In the first analysis (Fig. 2A), the higher recapture probabilities for females and immature males in the 14–18 mm size class relative to larger fish may thus have occurred because predators preyed selectively on equal-sized, but more conspicuous mature males. To confirm this, we compared recapture probabilities of mature males versus equal-sized

TABLE 3B. Analysis that includes mature males in the 14–18 mm size class.

Effect	df	χ^2	P-value
Recapture \times predator	1	29.50	<0.001
Recapture \times size class	3	84.13	<0.001
Recapture \times predator \times size class	3	2.25	0.52

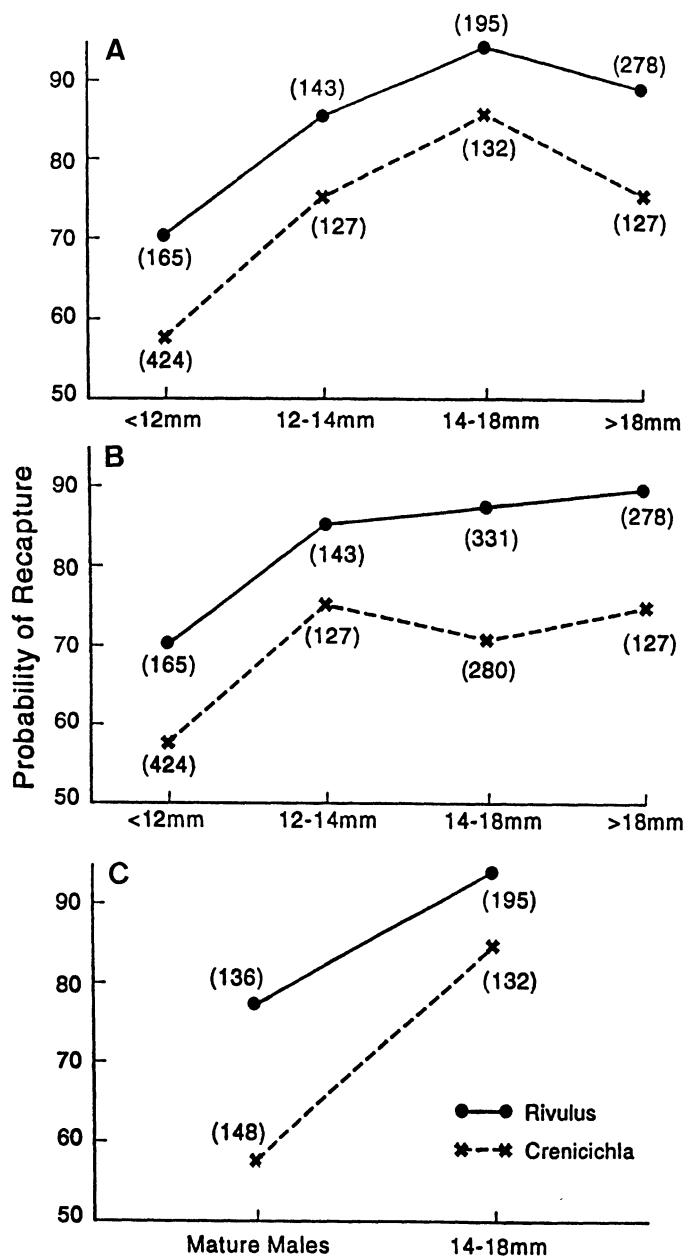


FIG. 2. (A) Recapture probabilities of juveniles, immature males, and females. We report here the average recapture rate of each size class for each type of community. Mature males are excluded from this analysis because they are brightly colored and have conspicuous courtship displays, two features that might influence susceptibility to predation. The numbers of individuals marked and released in each size class are indicated in parentheses next to each data point. The probability values are derived from a log-linear analysis with survival, size, and predator as independent variables. (B) Recapture probabilities as above, but with mature males included in the analysis. All other information is as in Figure 2A. (C) Recapture probabilities of mature males versus equal-sized females and immature males (14–18mm). All other information is as in Figure 2A.

females and immature males (Fig. 2C). The recapture probabilities of mature males were significantly lower than females and immature males in both types of localities ($\chi^2 = 44.5$, 1 df, $P < 0.001$). Again, recapture probabilities were

uniformly lower in *Crenicichla* localities than in *Rivulus* localities ($\chi^2 = 18.93$, 1 df, $P < 0.001$).

The probability of recapture was significantly lower in *Crenicichla* localities for all comparisons. If we consider these data as the mortality rate, rather than the probability of recapture (i.e., $1 - p[\text{recapture}]$), then the mortality rates of guppies from *Crenicichla* localities were more than twice as high for some size classes. However, the added increment of mortality in the *Crenicichla* localities was approximately equal for all size classes; all size classes experienced a 10–15% increase in total mortality per 12 d (Figs. 2A,B; three-way interaction in Table 1). Therefore the patterns of mortality in natural guppy populations were not those predicted by the age-specific mortality hypothesis.

Over the entire study, the proportion of marked animals that emigrated was 4.6% for *Crenicichla* and 4.9% for *Rivulus* sites. Therefore, it is unlikely that differential emigration is a source of bias in the estimated mortality rates in these two types of localities.

DISCUSSION

These results suggest that our long held hypothesis for the cause of guppy life-history evolution, age-specific differences among localities in mortality rates, may be incorrect. Our two main conclusions are: (1) guppy mortality rates are significantly higher in *Crenicichla* localities than *Rivulus* localities; and (2) these differences are uniformly spread across all size classes, rather than being size specific. It is the second conclusion that appears to contradict the age-specific mortality hypothesis. Additional conclusions are that juveniles suffer higher mortality rates than adults and that mature males suffer higher mortality rates than equal-sized females and immature males in all localities.

The Age-Specific Mortality Hypothesis

What accounts for the relatively low recapture probabilities of juvenile guppies in *Crenicichla* localities? This observation alone can account for the deviation of our results from the predictions of the age-specific mortality hypothesis (Fig. 1) and was not expected since *Crenicichla* were thought to prey selectively on large, adult size classes. While performing mark-recapture studies, we also surveyed invertebrate predators. We found that invertebrate predators like dragonfly and damselfly larvae were more abundant and larger at *Crenicichla* localities. These additional predators represent one possible cause of the lower than expected recapture rates of juveniles in *Crenicichla* localities. Mattingly and Butler (1994) also found that *Crenicichla* are not as narrowly size selective as previously assumed.

This pattern of juvenile mortality cannot be accounted for by the presence of juvenile *Crenicichla*. Juveniles were present in only one of our seven study pools during the course of our experiments. This pool had exceptionally low predation rates on all size classes of guppies, possibly because the young *Crenicichla* were still in the schooling stage and being guarded by their parents. At this stage, they feed primarily by pecking on the surfaces of rocks and logs and presumably are eating small invertebrates. The adults were

spending most of their time guarding their young from the school of *Astyanax* also found in the pool.

We do not know the actual causes of mortality in any of our study sites, since we rarely directly observed predation or other mortality events. We assume that predation was the primary cause of the increased mortality rates in the absence of any evidence for other causes of mortality, such as parasitism or disease. Other causes may not be size specific or might mask the size-specific preferences of predators if, for example, they resulted in a higher mortality rate of juveniles in a locality where predators preyed selectively on larger size classes. However, what counts for the evolution of life-history patterns is the overall mortality pattern experienced by guppies, which is what we have measured.

What mechanism accounts for the evolution of life-history patterns in guppies? Can the uniform difference in mortality rates implied by these results select for the observed patterns? The theoretical literature contains contradictory predictions on this matter. Some optimization models of age-specific mortality (e.g., Gadgil and Bossert 1970; Law 1979; Michod 1979; Roff 1980) predict that such a uniform change in mortality will not change the life history, which is inconsistent with our earlier observations of life-history evolution in guppies transplanted to a site with a different predator community (Reznick and Bryga 1987; Reznick et al. 1990). On the other hand, Koslowski and Uchmansky (1987) predict that a uniform reduction in survival will favor a decrease in the age at maturation and an increase in reproductive investment; this prediction is consistent with our results. One key difference among these models is Koslowski and Uchmansky's use of the lifetime production of offspring (R_0), rather than the intrinsic rate of increase (r) as an index of fitness. The structures of the models also differ in many other regards. Therefore, according to at least one theory, the observed uniform differences in mortality rate represent a sufficient mechanism for the observed life-history evolution.

It is also possible that a different way of accounting for the effect of juvenile mortality rates on fitness could alter our conclusions. We have dealt here with estimates of mortality rates, or the probability of dying in a fixed interval of time. This is the same variable used by theoreticians (e.g., Gadgil and Bossert 1970; Law 1979; Michod 1979; Charlesworth 1980) so we have evaluated the age-specific mechanism in a strict sense. However, it may be more valid to use the probability of survival to maturity, rather than juvenile mortality rate, to index the effects of predation early in life, as no contribution to fitness is made until an individual has the opportunity to reproduce.

We found that the guppies in *Rivulus* localities in these studies tend to grow more slowly than those in *Crenicichla* localities. Growth rates were estimated from the mean size of fish in a given millimeter size class at the time of marking and at the time of recapture; the difference between the two values is the two week growth increment. Slower growth delays maturity (Reznick 1982; Reznick and Bryga 1987; Reznick 1990). The combined effects of lower mortality rates but delayed maturity for juvenile guppies from *Rivulus* localities versus higher mortality rates and earlier maturity rates for juvenile guppies from *Crenicichla* localities potentially equalizes their probability of survival to first reproduction.

TABLE 4. Probability of survival to maturity for juvenile guppies from *Rivulus* and *Crenicichla* localities, using different values for standard length at birth and standard length at maturity.

Size at birth	<i>Rivulus</i>		<i>Crenicichla</i>	
	6 mm	7 mm	6 mm	7 mm
Size at maturity				
14 mm	0.1924	0.2362	0.1581	0.1896
15 mm	0.1578	0.1937	0.1517	0.1819
16 mm	0.1216	0.1466	0.1271	0.1524

We used the average growth and mortality rates in these two types of localities to calculate the probability of survival to maturity. To do this, we calculated the number of days it would take an individual to grow into the next size class and, given the probability of dying per day in a size class, we determined the probability that it would survive until it reached the next size class. One complicating feature of these calculations is that guppies from these two types of localities differ in the size at birth and the size at maturity. For example, guppies from *Crenicichla* localities are smaller at birth (~ 6 mm) and at maturity (~ 14 mm) than their counterparts from *Rivulus* localities (7 mm at birth and 16 mm at maturity). These differences are part of the evolved response to predation. We consider it most valid to compare probabilities of survival to maturity when birth size and size at maturity are equal for both types of localities so that the combined effects of the environment and predation are not confounded with the evolved response to these effects. The range of values that we considered for both variables spans the ranges that we have observed in natural populations. We found that the probabilities of survival to maturity were very similar for a given pair of sizes at birth and maturity and that the differences were always much smaller than would be inferred from a comparison of juvenile mortality rates alone (Table 4). For example, the mortality rate figures imply that approximately 30% of guppies < 12 mm long die per two-week period in *Rivulus* localities, as compared to 45% in *Crenicichla* localities (Fig. 2). In contrast, the mean probability of survival from birth to maturity, assuming a birth size of 6 mm and maturity at 15 mm (Table 4), is 15.8% for a *Rivulus* locality versus 15.2% for a *Crenicichla* locality. Again, the reason for this substantial reduction in the difference between the two is that guppies from the high predation localities also tended to grow faster, which results in an earlier age at maturity. Our direct estimates of adult mortality rates still argue that the probability of surviving to reproduce a second or subsequent time is considerably lower in a *Crenicichla* locality (approximately 20% mortality per 12 d versus 10% in a *Rivulus* locality: > 18 mm size class in Figs. 2A, B). The results for the relative mortality rates of mature males from each type of locality are similar (Fig. 2C), with the mature males from *Crenicichla* localities sustaining much higher mortality rates than their counterparts from *Rivulus* localities.

If these results for the probability of survival to maturity and adult mortality prove to be robust, then we would conclude that there is little or no difference between predator treatments in the probability of survival to maturity, but that guppies from *Crenicichla* localities suffer higher adult mor-

tality rates. Such results would be consistent with the age-specific predation hypothesis.

Alternative Mechanisms of Life-History Evolution

The difference among locality types in growth rates raises another possible mechanism for life-history evolution that would have to be considered whether or not the age-specific mortality hypothesis were supported by our results. This difference in growth rate is almost certainly attributable to confounding environmental rather than genetic causes. When these fish are reared in a common laboratory environment on controlled food availability, the somatic growth of guppies from *Rivulus* localities tends to be higher, rather than lower, than those from *Crenicichla* localities (Reznick 1983). Water temperatures are fairly uniform across these study sites, so this is not a likely cause of the differences in growth.

One possible cause of the differences in growth rates in natural populations is that they are an indirect consequence of predation. Higher survival across all age classes in *Rivulus* localities potentially results in increased population densities either in absolute terms or in terms of per capita resource availability than in *Crenicichla* localities. Such differences in density were observed in an earlier study (Reznick and Endler 1982). Higher population densities can result in lower growth rates. Similar indirect consequences of predation have been found in other aquatic ecosystems (e.g., Power 1987; Vanni 1987). A second possibility is that lower growth rates are caused by environmental differences that are independent of the predators, such as a tendency towards lower light levels and hence lower levels of primary productivity in *Rivulus* localities.

Differences in density and resource availability select for changes in the life histories of *Drosophila* (e.g., Bierbaum et al. 1989; Mueller et al. 1991, 1993); however there are no general predictions for how the life history will evolve in response to density effects, other than the traditional, but highly controversial, predictions from the theory of *r* and *K* selection (reviewed by Stearns 1992). The generally accepted consequences of increased density dependence are consistent with the observed guppy life-history patterns.

A second consequence of density effects is that they can interact with age-specific mortality patterns to influence the predicted evolution of the life history (Charlesworth 1980). For example, if density affects all age classes equally and if mortality rates are increased uniformly for all age classes, then it is predicted that there will be no evolutionary change in the life history. However, if density has a more adverse effect on juveniles than adults, then it is predicted that selection will favor individuals with earlier maturity and higher reproductive efforts. This latter prediction is consistent with the patterns that we have seen in guppies.

More generally, such a confounding of different forms of selection, rather than single, testable causes, has been observed in past investigations of evolution in natural populations (e.g., Jones et al. 1977; Price and Grant 1984) and may well be a common theme in the real world. Jones et al. (1977) conclude that eight different forms of evolution are responsible for a trait as simple as shell color and banding in the snail *Cepea nemoralis*. Three of these, background

color matching, apostatic selection, and the effects of shell color on body temperature, all fall under what we think of as natural selection. In our case, it seems very likely that the differences in predator communities and mortality rates will also affect population density, habitat utilization, and per capita resource availability. There are also average differences in the nature of the habitat in which each predator community is found. A complete understanding of guppy life-history evolution will certainly demand some evaluation of these indirect effects of predation and the influence of other environmental factors.

Implications for Other Aspects of Guppy Biology

Guppies have been widely used for investigations of the evolution of male color patterns, behavior, and sexual selection. Much of this work involves comparative studies on Trinidadian populations. Our results are relevant to this body of work because it makes assumptions about guppy mortality patterns and differences in mortality among the different communities.

Endler (1978) originally dealt with six different types of predator communities and arrayed them on a continuum of the intensity of predation on guppies. He evaluated the relative threat of each type of predator to guppies on the basis of stomach content analyses, direct observations of attack rates on guppies, and predator population densities. He postulated that the localities differed in the probability that an adult male guppy would be eaten per unit time. *Rivulus* localities were ranked as having the lowest intensity of predation and *Crenicichla* localities, which include other predators such as characins, were ranked as having the highest. Most of the intermediate localities contained different subsets of the predators found at *Crenicichla* localities. He found that male guppies were the most brightly colored in *Rivulus* localities and the least brightly colored in *Crenicichla* localities. He hypothesized that these differences represented a balance between sexual selection (females prefer brightly colored males) and natural selection (predators prey more readily on more conspicuous males). Our work confirms that, at least for the two extremes of his continuum, Endler's estimates of the relative mortality rates of male guppies were correct.

Other researchers report an association between the predator fauna (usually *Rivulus* versus *Crenicichla* localities) and guppy behavior patterns. Guppies from high predation localities have more highly developed predator evasion behaviors, including shoaling (e.g., Seghers 1974a,b; Farr 1975; Breden et al. 1987; Magurran and Seghers 1991; for a review, see Magurran et al. 1993). Female mate choice preferences vary among localities (Breden et al. 1987; Stoner and Breden 1988; Houde and Endler 1990) as do levels of aggression in food patch defense (Magurran and Seghers 1991). There are also differences in male guppy sexual behavior between predator localities (Farr and Herrnkind 1974; Farr 1975; Luyten and Liley 1985; Magurran and Seghers 1990, 1994) but some of these may result from indirect effects of predation, such as differences in guppy population demography (e.g. sex ratio and density), rather than direct responses to predation (Seghers 1973; Reznick and Endler 1982; Rodd and Sokolowski, in press; Rodd and Reznick, in press). All of this work has

assumed that these localities differ in male mortality rates or overall mortality rates, as predicted by Endler (1978). Again, our results validate these assumptions.

CONCLUSIONS

While the simplest interpretation of our results rejects the age-specific mortality hypothesis, other interpretations of the new information have expanded, rather than reduced, the possible mechanisms that might be causing the evolution of life histories in guppies; age-specific differences in mortality remains only one of the viable mechanisms. More generally, while the specific mechanism(s) of natural selection remain uncertain, such large differences among localities in mortality rate are the likely cause of this life-history evolution. These patterns are well correlated with the predators with which guppies coexist, suggesting that predators are the ultimate cause of these differences in mortality rates.

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