Ecology, 78(5), 1997, pp. 1370–1381

RESOURCE AVAILABILITY AND PLASTICITY IN OFFSPRING PROVISIONING: EMBRYO NOURISHMENT IN SAILFIN MOLLIES

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Abstract. I report evidence of plasticity in the mode of embryo nourishment by female poeciliid fish raised under contrasting environmental conditions. In two experiments, female sailfin mollies (*Poecilia latipinna*), raised on high and low levels of food, produced neonates of similar mass and percentage of fat by varying egg size and the amount of supplemental nourishment provided to embryos as they developed. In one experiment, females displayed plasticity in ovum size, but not neonate size; females raised in a low-food and low-salinity treatment produced larger eggs than those raised on higher food levels and at higher salinity. In a second experiment, the amount of nourishment provided to embryos, in addition to that in the egg yolk, was dependent on brood size and maternal food level. Females with small broods were less matrotrophic than those with large broods; female body size and brood size were highly correlated. The brood size at which egg mass equaled neonate mass was smaller for females raised on low levels of food than for females raised on a higher food level treatment. In the second experiment, females from two populations, different from the source of fish for the first experiment, were studied and found to differ in the amount of fat stores remaining after reproduction. Females from a population with low postparturition fat stores displayed greater brood reduction during gestation (via resorption or abortion) and fewer offspring per unit mass than females from the population with more fat. In sailfin mollies, matrotrophy appears to be an adaptation that diminishes the offspring size–offspring number trade-off by permitting a reduction in ovum size and increase in fecundity without compromising neonate size. However, matrotrophic supplementation of yolk nourishment was greatest in relatively large females raised on a restricted food level. Thus, matrotrophy may incur some energetic cost that renders it inefficient for small females and for females with substantial or dependable energy reserves available for reproduction.

Key words: energy storage; fecundity; lecithotrophy; life history; matrotrophy; phenotypic plasticity; Poecilia latipinna; *reproductive investment; resource availability; scope for reproduction; trade-off; viviparity.*

INTRODUCTION

The level and duration of parental investment is thought to be a function of available resources and the effects of investment on future survival (Roff 1992, Stearns 1992). Offspring-provisioning models illustrate that variations in resource type (McGinley and Charnov 1988), resource availability during provisioning (Lloyd 1987, McGinley et al. 1987), and resource predictability (Lalonde 1991) influence the optimal level of reproductive investment. Because all of these forms of resource variability may be experienced by natural populations, it should be no surprise that plasticity in reproductive investment is widespread.

One idealized pattern of reproductive investment, in a variable environment, is for females to produce a large number of embryos, with minimal investment in each embryo until as late in their developmental cycle as possible. At a critical point, excess offspring should be resorbed and the remaining offspring brought to term at equal quality (Kozlowski and Stearns 1989). Selective brood reduction, when feasible, should also favor production of excess zygotes without regard to environmental conditions, in anticipation of aborting those with lowest fitness (Lloyd 1980, Stearns 1987). Brood reduction is well documented in plants and animals, although it appears that females of some species have limited ability to abort offspring late in development (Lalonde and Roitberg 1989). Several authors have suggested that brood reduction by embryo resorption or abortion takes place in poeciliid fishes (Scrimshaw 1944, Turner 1947, Schultz 1961, Hester 1964, Borowsky and Kallman 1976), but Meffe and Vrijenhoek (1981) failed to find experimental evidence for embryo resorption.

One factor related to plasticity in female reproductive investment patterns is the source of nutrients used to nourish developing embryos (Reznick and Yang 1993, Reznick et al. 1996). Nutrients may come from resources stored in a reservoir of somatic fat, or they may be recently acquired and rapidly mobilized (Stearns 1992:80). Females that draw on stored nutrient reserves may be more buffered from the vagaries of

Manuscript received 29 May 1996; revised 16 September 1996; accepted 13 November 1996; final version received 5 December 1996.

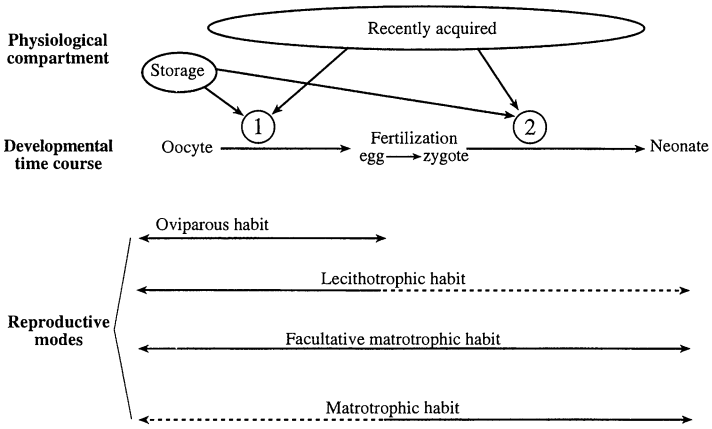

FIG. 1. A conceptual model of embryo nourishment patterns. Physiological compartment refers to the source of nutrients and energy expended during reproduction by females. Energy expenditure from either compartment may occur during developmental periods 1 or 2, and the size of each pool may vary. Oviparous fish can only contribute during period 1, whereas highly matrotrophic species (those producing eggs with little yolk) contribute primarily during period 2, and lecithotrophic species contribute primarily during period 1. Facultatively matrotrophic species may spread their contributions between periods 1 and 2, or shift their investment primarily to one or the other period, depending on conditions. The maximum absolute volume of the storage compartment is correlated with body size.

environmental conditions than females that rely on recent acquisitions. For example, the extended maternal care of viviparous species, if fueled by stored resources, may permit extensive scope for plasticity in the level and timing of maternal investment. In contrast, oviparous reproduction, fueled by recently acquired resources, permits less scope of variation in the flow of resources to reproductive investment (Fig. 1).

In live-bearing fishes, retention of embryos permits prolonged opportunity for their nourishment by the mother and may serve to spread out the reproductive investment period, postpone the final commitment to offspring quality, and permit expanded options of plasticity in reproductive investment (Thibault and Schultz 1978). Females that brood their embryos internally can alter the size of offspring they produce by varying their initial contribution of yolk to eggs, or by supplementing the yolk as the embryos develop (Scrimshaw 1944, 1945, 1946). Variation occurs among species in the relative contribution of yolk-derived (lecithotrophic) and maternally derived (matrotrophic) nourishment supplied to developing embryos (Wourms et al. 1988). Environmentally mediated embryo nourishment in fish was hypothesized by Thibault and Schultz (1978), and Gilmore (1983) has demonstrated that some sharks seasonally supplement their lecithotrophic embryo nourishment by production of trophic eggs. Adelphophagy and oophagy, the feeding of embryos on developing siblings or ova, have been indicated for a number of fishes (Wourms et al. 1988), including goodeids (Greven and Grossherr 1992), but without evidence of maternal or environmental mediation. Evidence for environmentally mediated embryo nourishment in poeciliids was first reported from field-collected sailfin mollies, *Poecilia latipinna* (Trexler 1985). Facultative embryo nourishment has also been described for several species of snakes (Stewart 1988).

In this paper, I report two laboratory experiments designed to test the role of resource availability in reproductive plasticity by varying the scope for reproduction in female sailfin mollies. Scope for reproduction is the amount of energy that is available for reproduction beyond that required for maintenance and growth (Brett 1979). These studies test the hypothesis that embryo nourishment pattern is a plastic response to female energetic status, and they provide evidence for extreme plasticity in reproductive investment via embryo nourishment in this species.

MATERIALS AND METHODS

Overview of the experimental protocol

The role of matrotrophy in embryo nourishment was determined experimentally by comparing the mass of early-stage embryos (compact-blastula stage; Tavolga and Rugh 1947) and neonates. The change in mass between these two stages is indicative of the relative amount of matrotrophic contribution to developing embryos, i.e., the less mass lost during development, the greater the maternal contribution in addition to that in the yolk (Hoar 1969, Thibault and Schultz 1978). Loss of 35–50% mass or more is observed for egg-laying (oviparous) species (Wourms et al. 1988).

Blastula mass was measured as an indicator of ovum mass. It was necessary to obtain mass measurements after fertilization to assure that yolk loading was complete (Snelson et al. 1986). For experiment 1, dry mass was obtained by drying tissues in a freeze-dryer; for experiment 2, tissues were dried in an oven at 55°C. In both cases, drying was continued in 24-h intervals until a constant mass was obtained three consecutive times. Prior to drying, we measured ovum diameters from formalin-preserved ova at the blastula stage, using a compound microscope with an ocular micrometer. Formalin preservation was necessary because unpreserved ova cannot be separated intact from the ovarian tissues. There is no effect of formalin preservation on ovum mass (Scrimshaw 1945, Thibault and Schultz 1978; J. C. Trexler, *personal observation*). Analyses indicated that ovum diameter added no additional information beyond that derived from analysis of mass, so I report only results of mass analyses.

In both experiments, environmental conditions were varied with the intent of influencing female scope for reproduction, but the conditions used differed. The first experiment varied both salinity and food level, whereas the second varied only food level. This change was purely logistical. Studies prior to experiment 1 failed to indicate a food level that would affect reproductive scope, but would not shut down reproduction completely (Trexler and Travis 1990; J. C. Trexler, *unpublished data*). By the start of experiment 2, such food levels were identified and employed as the sole experimental variable. Variation of a single factor was considered preferable once it became feasible. Sample sizes could not be well controlled in either study, because it was not possible to separate female mollies from males at the outset of rearing. Variation in interbrood sex ratio led to unequal sample sizes.

Experiment 1

Salinity and food level were varied in concert in an effort to maximize the difference between the low and high scope-for-reproduction treatments. Female sailfin mollies raised on a constant food level mature at a relatively smaller size and later age in low-salinity water (0–2 g/kg), compared to those raised at higher salinity (6–24 g/kg) (Trexler and Travis 1990). Mortality rates are generally higher in freshwater than in saltwater, both in the field (Trexler et al. 1992) and the laboratory (J. C. Trexler, *unpublished data*). Thus, freshwater conditions are more physiologically demanding for this species than saltwater ones. This is supported by studies of the osmotic mechanisms of sailfin mollies, which are more like those of marine than freshwater fishes (Evans 1973, 1975).

Thirty-three female mollies were raised singly, from birth to sexual maturity, in 18.9-L aquaria under controlled feeding and salinity regimes. These fish were offspring of field-collected females maintained in isolation in the laboratory, under standardized conditions, until the birth of their offspring. Only one female offspring per field-collected female was used in the experiment, to minimize bias from family-level variation (Trexler and Travis 1990). The low-scope treatment females received less than one-half of the daily ration of the high-scope treatment (Fig. 2), and the low-scope treatment was maintained at 1 g/kg salinity, whereas the high-scope was maintained at 14 g/kg. Temperature within the aquarium room was maintained at 27°C. Excess food was removed from each tank daily. When a female reached sexual maturity, two male mollies were added to the tank. At the outset of the experiment, fish were assigned to one of two treatments: (1) sacrifice 14 d after addition of males, in order to measure the size of embryos at the blastula stage; or (2) sacrifice immediately after the birth of offspring. All fish were sacrificed by rapid freezing, following guidelines for humane care of laboratory fishes. Fish raised for this experiment were obtained from female mollies collected from marshes on the southern shore of Tampa Bay, Florida, United States (FL-TB).

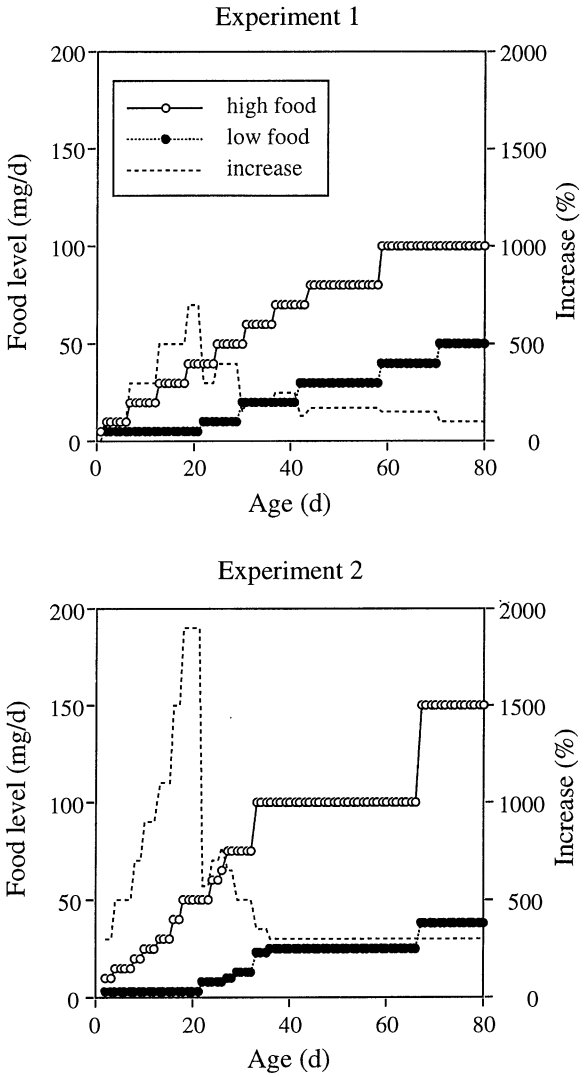

FIG. 2. Plots of daily rations fed to sailfin molly fish during each experiment. The dashed "increase" lines show the percentages (right-hand vertical axes) by which high food levels exceed low food levels at each age: 100 × (high − low)/low. In experiment 2, the maximum daily ration was fed in two portions, one in the morning and one in the late afternoon.

Experiment 2

This experiment followed a design similar to that of experiment 1 with the following exceptions. Fish were collected from two source populations, one near Georgetown, South Carolina (SC), and one from Saint Marks, Florida (FL-SM), United States. Field-caught females and their offspring were maintained in 37.8-L aquaria at 6 g/kg salinity throughout the study. To increase sample size over that of experiment 1, four sibling fish from the first laboratory brood of each female were raised from birth to sexual maturity in one tank.

Males were removed from the group as soon as they could be identified; as each female matured, she was moved into a separate tank with two males. From this design, 90 female mollies were obtained. Food level was varied to a much greater extreme than in experiment 1. High-food-level fish were fed a ration twice daily, whereas low-food-level fish were fed a much lower ration only once daily (Fig. 2). Females were sacrificed at the end of each treatment, and the mass of their lean tissues, minus intestine and ovary, and the mass of fat present in those tissues was determined. Fat was extracted by use of petroleum ether (Kerr et al. 1982, Dobush et al. 1985) and mass was determined by difference before and after extraction. Fat was also extracted from blastulae and neonates, and lean dry mass was measured. Fat stores were analyzed for 82 females and their broods; eight females were damaged in tissue preparation and could not be used reliably.

Statistical analyses

Statistical analyses employed standard techniques of ANOVA, ANCOVA, and backwards stepwise linear regression. Preliminary examination of the data led to transformation of all variables as their natural logs, to better fulfill the assumptions of normal-theory analyses. All analyses used the type III calculation of sums of squares and all factors were considered fixed (Sokal and Rohlf 1981). I report coefficients of determination (CD) for all significant terms. With type III sums of squares, the CD indicates the percentage of total variation *uniquely* attributable to each factor in the model. This is a conservative approach, because additional variance may be attributable to multiple factors, i.e., multicolinearity; these CDs do not sum to the model's R^2 value. Backwards stepwise linear regression begins with analysis of a saturated model, and is followed by sequential elimination of the single nonsignificant factor that explains the least variance, until a model containing only significant factors is identified. This approach was employed to minimize multicolinearity in our final models. Analyses were carried out using SYSTAT (Wilkinson 1988).

Reproductive allocation is commonly analyzed as the ratio of reproductive output to female mass (Roff 1992). However, analysis and interpretation of such ratios must be done with caution (Packard and Boardman 1987, Roff 1992). Difference in reproductive allotment between treatments could indicate a deviation from isometry in reproductive output, or a difference in the magnitude of reproductive allocation, or both. These points can be disentangled by analysis of covariance of reproductive allotment between treatments, with female mass as a covariate (Packard and Boardman 1987: Fig 10.2). Additional concerns in such analyses arise from small correlations of ratios with their denominator (Praire and Bird 1989, Klinghamer et al. 1990, Roff 1992:132–134). I analyzed reproductive allotment as the difference of the natural logs of the dry mass of reproductive tissues and female dry mass (soma lean mass plus fat) (Mosimann and James 1979). Female dry mass was included as a covariate in statistical analyses, but interpretations focus on the effects of feeding treatment, developmental stage, and source population. Care was taken to avoid interpreting small correlations of reproductive allocation and female dry mass, because dry mass is the denominator of reproductive allocation (Roff 1992). In the single instance in which the correlation of a ratio with its denominator was of interest, bootstrapping was used to adjust for any resulting bias. This approach was chosen because any artifactual correlation of denominator and numerator is also present in the sampling distribution used to estimate type I error rate.

RESULTS

Experiment 1

The high- and low-scope treatments of this experiment did not produce females that matured at different sizes, although their fecundity was quite different. The mean dry mass of low-scope females was 146.8 mg (N = 22) and the mean dry mass of high-scope females was 147.8 mg (N = 11) (Treatment $F_{1,29}$ = 0.01, P = 0.94). The low-scope treatment was not so rigorous as to limit female growth; females on both treatments continued to grow during gestation, and those that carried their embryos to parturition were 30.7% heavier (168.5 mg) than those sacrificed at the blastula stage (126.8 mg) (Developmental stage $F_{1,29}$ = 10.863, P = 0.003; Treatment × Developmental stage $F_{1,29}$ = 0.101, P = 0.753). The mean brood size (measured as number of embryos at the blastula stage or number of offspring at the neonate stage) of low-scope females, adjusted for body size, was 7.2 and that of high-scope females was 12.7, a 76% difference (Treatment $F_{1,28}$ = 2.571, P = 0.006). Brood size did not differ between blastula-stage and neonate-stage broods (Developmental stage $F_{1,28}$ = 0.346, P = 0.288).

In this experiment, low scope-for-reproduction females produced ova that lost, on average, 33.7% of their mass prior to birth, whereas high scope-for-reproduction females produced ova that lost only 8.9% of their mass (based on adjusted means, Table 1). This difference arose because neonate sizes were comparable between treatments, but blastulae from low-scope females were 21% larger than those from high-scope females. This difference in development between the food treatments is revealed by the significant interaction of treatment and developmental stage (Table 1). When each developmental stage was analyzed separately, treatment level did produce a significant difference at the blastula stage (ANCOVA: Treatment $F_{1,12}$ = 5.357, P = 0.039; Brood size $F_{1,12}$ = 12.086, P = 0.005; Treatment × Brood size $F_{1,12}$ = 2.741, P = 0.124). However, treatment was not significant at the neonate stage (ANCOVA: Treatment $F_{1,12}$ = 0.319, P = 0.582; Brood size $F_{1,12}$ = 1.302, P = 0.275; Treat-

TABLE 1. Analysis of blastula and neonate size produced by female sailfin mollies raised under two conditions of scope for reproduction in experiment 1. Adjusted means were compared at the log-transformed grand mean brood size of 2.167 offspring (untransformed 8.73).

A) ANOVA of blastula and neonate size

Source	SS	df	MS	F	P	CD†
Treatment	0.005	1	0.005	0.259	0.615	
Developmental stage	0.427	1	0.427	20.377	0.000	23.0
Treatment × Stage	0.168	1	0.168	8.027	0.008	12.1
Brood size	0.225	1	0.225	10.754	0.003	9.1
Error	0.587	28	0.021			

B) Adjusted mean blastula and neonate size

Food level	Developmental stage	Adjusted means (mg) Transformed (±1 SE)	Untransformed	N
Low	blastula	−5.505 (0.050)	4.07	9
High	blastula	−5.695 (0.056)	3.36	7
Low	neonate	−5.916 (0.041)	2.70	13
High	neonate	−5.790 (0.078)	3.06	4

† Coefficient of determination (see *Methods: Statistical analyses*).

ment × Brood size $F_{1,12} = 0.640$, $P = 0.438$). In summary, low-scope females produced relatively few young that lost mass during development, whereas high-scope females produced 76% more babies that lost little mass during development. Low-scope females must have relied more on lecithotrophy to nourish embryos than did high-scope females.

Reproductive allotment was greatest for females raised on the high scope-for-reproduction treatment, and at the blastula development stage. The dry mass of reproductive tissues from females with embryos at the blastula stage was, on average, 23.2% of the somatic dry mass of low-scope females and 27.6% of that of high-scope females. This contribution to reproduction declined to 12.1% and 26.2% for low- and high-scope treatments at parturition, respectively (ANCOVA: Treatment $F_{1,29} = 5.901$, $P = 0.022$; Developmental stage $F_{1,29} = 3.271$, $P = 0.081$; Treatment × Stage $F_{1,29} = 2.387$, $P = 0.13$).

Experiment 2

The food level treatment in this experiment produced females that matured at different sizes. The mean dry mass of females raised on the low-food treatment was 204.9 mg and that of females raised on the high-food treatment was 480.5 mg, a greater than twofold difference. As in experiment 1, females continued to grow during gestation; there was a mean 24.5% difference in mass between blastula-stage females and parturition-stage females. There was no difference in mean dry mass of females resulting from their population source (ANOVA: Food treatment $F_{1,78} = 75.282$, $P < 0.0001$; Developmental stage $F_{1,78} = 4.973$, $P = 0.029$; Population source $F_{1,78} = 1.736$, $P = 0.191$; no significant interactions). Females from the SC population tended to have more fat than those from FL-SM. The size-adjusted mean percentage of fat for females raised in the high-food treatment, pooled across source populations, was 23.7%; for the low-food treatment, it was 14.4%. The SC females averaged 20.6% fat, whereas those from FL-SM were 17.5% fat (percentage fat: Food treatment $F_{1,78} = 34.329$, $P < 0.001$; Population source $F_{1,78} = 6.749$, $P = 0.012$; Female dry Mass $F_{1,78} = 4.394$, $P = 0.04$; no significant interactions). Percentage of fat did not change during gestation.

The low-food females produced smaller brood sizes, adjusted for female mass, but the effect was greatest for the fish from FL-SM (Table 2, Fig. 3). Also, brood size was different for the two developmental stages. It was lower for the neonate stage in all combinations of source population and food level, with a large decrease in the FL-SM population at both food levels and in the SC population at the high-food level (Table 2, adjusted means). This analysis is hampered by the small sample size of neonate broods. However, the observation of smaller brood size at parturition than at fertilization is supported by reanalysis pooling the two populations. Larger females produced fewer offspring per unit mass than did smaller ones (Table 3), although the correlation was weak (bootstrapped correlation of number of offspring per unit female mass and female mass, $r = -0.285$, $P = 0.006$).

Consistent with experiment 1, blastula mass was affected by the feeding treatment in experiment 2, when adjusted for brood size, but neonate mass was not (Treatment × Stage interaction, Table 4). The source population was also a significant factor, through an interaction with other effects. The adjusted means indicate that, on the high-food treatment, females from either population that produced 32 offspring (the grand mean) had offspring that lost 14.3% of their mass during development, averaged across source populations. On the low-food treatment FL-SM fish produced offspring that lost 9.2% of their mass and SC fish produced

TABLE 2. Analysis of brood size produced by female sailfin mollies from two source populations, raised under two feeding levels in experiment 2. Adjusted means were estimated at the log-transformed grand mean female mass of −1.099 (untransformed 333.0 mg). In (B), developmental stages are abbreviated B (blastula) and N (neonate).

A) ANOVA of brood size

Source	SS	df	MS	F	P	CD
Treatment	1.447	1	1.447	7.256	0.009	3.6
Source population	1.079	1	1.079	5.410	0.022	2.7
Developmental stage	2.618	1	2.618	13.126	0.000	6.5
Treatment × Source	0.953	1	0.953	4.779	0.032	2.4
Maternal mass	4.084	1	4.084	20.476	0.000	10.2
Error	16.754	84	0.199			

B) Adjusted mean brood size

Food level	Source†	Adjusted means: Transformed (±1 SE) B	Transformed N	Untransformed B	Untransformed N	Change (%), B − N	N B	N N
Low	FL-SM	3.199 (0.116)	2.806 (0.184)	24.5	16.5	−32.7	18	7
Low	SC	3.553 (0.180)	3.452 (0.227)	34.9	31.6	−9.5	8	4
High	FL-SM	3.774 (0.118)	3.491 (0.172)	43.6	32.8	−24.8	16	8
High	SC	3.870 (0.110)	3.361 (0.143)	47.9	28.8	−39.9	18	11

† FL-SM refers to fish collected from Saint Marks, Florida; SC indicates fish collected from Georgetown, South Carolina.

offspring that increased in mass by 14.3% (Fig. 4). The actual difference in mass loss depends on the brood size at which the comparison between treatments is made. This contrasts with experiment 1, in which high-food offspring lost less mass during development than did low-food offspring.

When analyzed separately, feeding treatment had a much greater effect on the mass of blastula-stage embryos than of neonates (Fig. 4). Tukey's hsd pairwise comparisons indicated that blastula-stage embryos differed in size under the high- vs. low-food treatments for both the SC and FL-SM populations (SC: $F_{1,76}$ = 4.452, P = 0.04; FL-SM: $F_{1,76}$ = 7.076, P = 0.009). Neonate masses did not differ between the two food treatments for either population (SC: $F_{1,76}$ = 3.741, P = 0.099; FL-SM: $F_{1,76}$ = 2.313, P = 0.264). The apparent difference between food levels in adjusted neonate mass for the FL-SM population was not significant, because the standard error was large for neonates from low-food females (−6.332 ± 0.158 mg, adjusted mean ± 1 SE) and the sample size was small. Unadjusted mean neonate mass was quite similar between food treatments (Fig 4).

Reproductive allocation was greatest for females raised in the high-food-level treatment, particularly at the blastula stage (Fig. 5). The differential effect of food level on reproductive allocation for the two stages of development explained more variation than did any other single effect (Table 5). Within treatments, reproductive allocation declined as female size increased (the slope for the effect Female dry Mass was negative). Differences between the source populations were only noted through their interactions with other effects, and they explained little variation. In fact, the full model explained only ≈30% of the total variation in reproductive allocation.

SC females produced blastulae with more fat than did females from FL-SM on the same food treatment. However, this population difference was diminished at the neonate stage (Source × Stage interaction, Table 6). Brood size did affect offspring fat stores, although its influence varied between the developmental stages (Stage × Brood size interaction, Table 6); generally, offspring from larger broods had less fat than those from smaller ones. There was no statistically significant effect of food-level treatment on the percentage of fat of blastulae or neonates.

DISCUSSION

Overview

Both experiments provided evidence that the amount of matrotrophic supplementation varied with the brood size and feeding history of the mother. This was indicated by a difference in the amount of mass lost during development by embryos brooded by females raised under contrasting environmental conditions. The first experiment indicated that females raised under conditions generating a high scope for reproduction contributed more matrotrophic nourishment to their developing embryos than did those raised under leaner conditions; the second experiment generated the opposite pattern from two other populations. There are a number of differences between the two experiments that could be responsible for apparent conflict in their results, including genetic differences in reproductive investment by the fish studied, size differences in the fish studied, and differences in the environmental conditions created in the laboratory. In the following overview, I will suggest that the observed patterns of embryo nourishment resulted from brood size variation, a correlate of female size, and from an interaction of the effect of brood size with maternal fat storage.

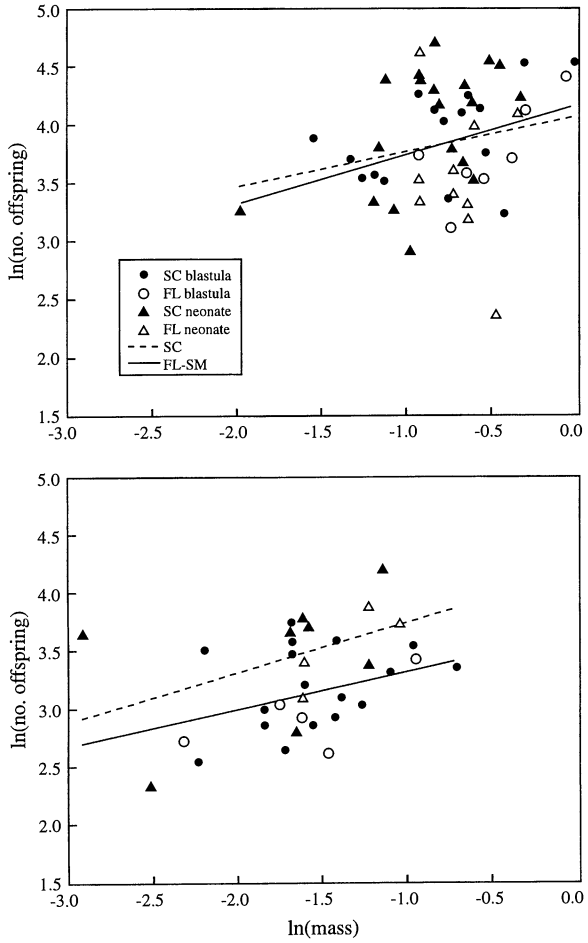

FIG. 3. Fecundity of females raised on high vs. low scope-for-reproduction treatments from experiment 2. The top panel illustrates the results for females raised on the high-food level, and the bottom panel illustrates results for low-food females. Codes for source populations are: SC, fish collected in South Carolina; FL or FL-SM, fish collected in Saint Marks, Florida.

I developed an index of matrotrophic contribution to embryo maturation by calculating the difference between the predicted size of blastula-stage embryos and the predicted size of neonates at the same brood size. At zero, this index indicates that the amount of matrotrophic contribution matches the depletion of yolk reserves required for development. As the index increases above zero, the amount of matrotrophic contribution increases, and as it decreases below zero, matrotrophic nourishment is less important. The percentage loss of mass for pure lecithotrophic development is unkown, but embryos from low-food females from experiment 1 approximated the mass loss in some oviparous fishes.

For all three populations studied in the two experiments, the matrotrophy index increased as brood size increased (Fig. 6, top panel). In experiment 2, the gain in matrotrophy with increasing brood size was greater for the SC population than for the FL-SM population. Since these fish were raised in a common-garden experiment, these results suggest a genetic difference between these two populations (maternal effects were not controlled, but were unlikely to be of consequence for such a character). Blastula and neonate size were approximately equal at a brood size of 20 ($e^{3.0}$) at the low-food level, but at the high-food level, the point of equity arose at a larger brood size, about 67 ($e^{4.2}$, Fig. 6, top panel). For the FL-TB females raised in experiment 1, the low-food females were less matrotrophic than the high-food females at all brood sizes.

Experiment 2 also provided evidence for a genetic difference in the effect of food level on brood size. At the high-food level, both SC and FL-SM females produced similar brood sizes, but at the low-food level, FL-SM females produced fewer offspring than did SC fish; similar-sized SC females produced approximately the same brood size at both food levels. The size of neonates produced by SC females was also larger at the low-food level (greater lean mass) than that of FL-SM neonates. Apparently, SC females have accomplished this by boosting their reproductive allocation at low-food levels, compared to FL-SM females, and by providing more matrotrophic supplementation to developing embryos than do FL-SM females. I found evidence of brood reduction after fertilization in the FL-SM and SC populations, but not in the FL-TB population. In experiment 2, brood reduction was least in the SC population at the low-food level, although the result must be viewed with caution, as only four females from the SC population were reared on the low-food level. This supports Meffe and Vrijenhoek's (1981) conclusion that female condition has little effect on embryo resorption in poeciliids.

Facultative matrotrophy

The most general result of this study was an increase in matrotrophy with increasing brood size. Larger females produced larger brood sizes and were more matrotrophic than small females. Experiment 2 indicated that low food led to matrotrophy at a smaller size than in the high-food treatment. This may explain the different results of experiments 1 and 2. Experiment 1 employed a population with a small average size, and low-food females were less matrotrophic than those raised on high food. If the matrotrophic indices for high- and low-level food cross at a brood size greater than that of the FL-TB population, the results of experiment 1 could be explained (Fig. 6, bottom panel). However, it is also possible that the salinity treatment of experiment 1 led to a different outcome from that of experiment 2, or that the response of the FL-TB population was different. Only a common-garden experiment could resolve this issue.

The increase in matrotrophy with size may be linked to the source of resources used in reproduction (Fig. 1) and to the cost of reproduction. Smaller female mollies have a smaller pool (absolute size) of stored resources than do larger females, even if their percentage

TABLE 3. Analysis of brood size per milligram of female mass [log(brood size) − log(female mass)] produced by female sailfin mollies raised under two feeding levels in experiment 2. Adjusted means were estimated at the log-transformed grand mean female mass of −1.099 (untransformed 333.0 mg). Only the significant model terms are reported. In (B), abbreviations are B = blastula and N = neonate.

A) ANOVA of brood size per mg female mass

Source	SS	df	MS	*F*	*P*	CD
Treatment	1.635	1	1.635	8.071	0.006	6.8
Source population†	1.019	1	1.019	5.032	0.028	4.2
Developmental stage	1.834	1	1.834	9.055	0.004	7.6
Treatment × Source	0.848	1	0.848	4.193	0.044	5.5
Mass	3.162	1	3.162	15.609	<0.001	13.1
Error	16.004	79	0.203			

B) Adjusted mean brood size per mg female mass

Food level	Source†	Adjusted means: Transformed (±1 SE) B	Transformed (±1 SE) N	Untransformed B	Untransformed N	*N* B	*N* N
Low	FL-SM	−2.659 (0.124)	−2.899 (0.213)	0.070	0.055	18	7
Low	SC	−2.278 (0.183)	−2.362 (0.229)	0.102	0.094	8	4
High	FL-SM	−2.017 (0.119)	−2.315 (0.187)	0.133	0.099	16	8
High	SC	−1.924 (0.111)	−2.427 (0.152)	0.146	0.088	18	11

† Source population codes are as in Table 2.

of fat is the same. Thus, smaller females may depend more heavily on resources acquired during egg yolking and gestation than on stored resources. In a variable environment, small females may be more susceptible than larger females to the vagaries of resource levels in completing the development of their embryos. The cost of individual offspring is greater for small females than larger ones, especially given that they produced neonates equal in size to those of larger females. Large females, with their relatively larger brood sizes, spread their investment in offspring over more of the gestation period than do smaller ones. My data do not permit determination of the energy pool (storage or recent acquisition) used to fuel reproduction.

Females continued to grow during gestation in both experiments and in all treatments; in experiment 2, they did not change in percentage of fat during gestation. This suggests that continued somatic investment is important to fitness in young females. Clearly, none of the low-scope treatments was restricted enough to severely limit reproduction and growth, although substantial differences in female size and/or brood size

TABLE 4. Analysis of the mean size of offspring at the blastula and neonate developmental stages under the two different feeding treatments of experiment 2. Source refers to the source population. Adjusted means were estimated at the log-transformed grand mean female mass of −1.099 (untransformed 333.0 mg). Only the significant model terms are reported.

A) ANOVA of offspring size

Source	SS	df	MS	*F*	*P*	CD
Treatment	1.144	1	0.144	10.279	0.002	9.9
Developmental stage	1.308	1	1.308	11.758	0.001	11.3
Treatment × Stage	2.771	1	2.771	24.905	<0.001	23.9
Source × Stage	0.620	1	0.620	5.571	0.021	5.4
Stage × Brood size	1.292	1	1.292	1.615	0.001	11.2
Stage × Source × Brood size	0.685	1	0.685	6.155	0.0155	5.9
Error	8.456	76	0.111			

B) Adjusted mean offspring size

Food level	Source†	Stage	Adjusted means (mg): Transformed (±1 SE)	Adjusted means (mg): Untransformed	*N*
Low	FL-SM	blastula	−6.237 (0.097)	2.0	17
Low	FL-SM	neonate	−6.332 (0.158)	1.8	7
Low	SC	blastula	−6.165 (0.139)	2.1	7
Low	SC	neonate	−6.014 (0.181)	2.4	4
High	FL-SM	blastula	−5.687 (0.099)	3.4	15
High	FL-SM	neonate	−5.824 (0.130)	3.0	8
High	SC	blastula	−5.611 (0.094)	3.7	18
High	SC	neonate	−5.818 (0.121)	3.0	9

† Source population codes are as in Table 2.

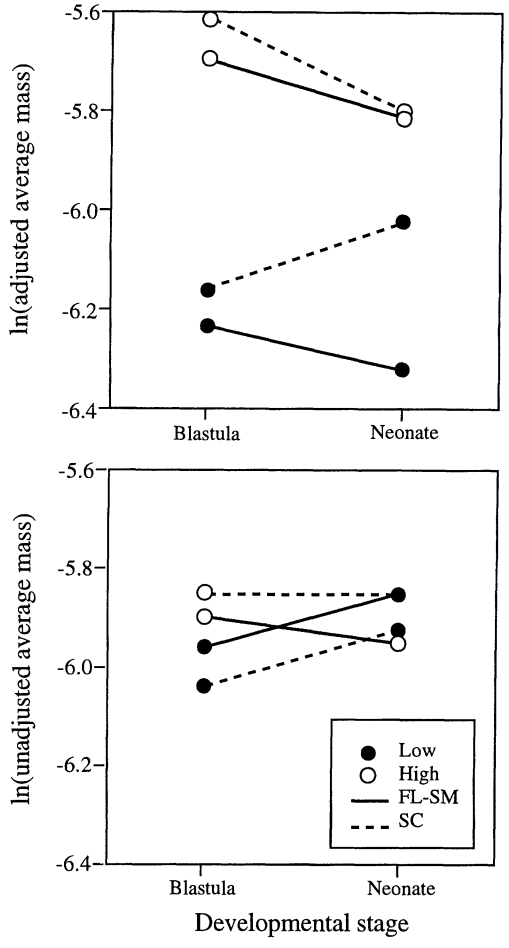

FIG. 4. Observed and adjusted blastula and neonate mass. The top panel illustrates the adjusted means of blastula and neonate size, according to feeding treatment and source population (see Fig. 3 codes). Adjusted means were compared at the log-transformed grand mean brood size of 3.493 offspring (untransformed 32.9 offspring). The bottom panel shows the observed means of blastula and neonate size according to feeding treatment and source population. The two panels differ because brood sizes of the FL-SM population on the low-food treatment were smaller than for other treatment combinations.

were noted in both experiments. Also, the treatments in experiment 2 produced realistic phenotypes. Females raised in the high- and low-food treatments of that experiment had percentages of fat comparable to those of field-collected females reproducing in early spring and midsummer, respectively. These levels matched the maximum and minimum mean percentage of fat observed (Ricci 1994). All data reported here were from the females' first reproductive bouts; future research must examine females producing second or later broods, to determine if growth–reproduction trade-offs are resolved differently later in life.

Reproductive investment

Reznick and colleagues have proposed a general model of offspring quality and reproductive investment for poeciliid fishes (Reznick and Yang 1993, Reznick et al. 1996). Assuming that a female's environment predicts the environment experienced by her offspring, they proposed that neonate size and energy stored as fat are critical determinants of survival in poor environmental circumstances. However, in more benign conditions, they proposed that offspring size is less critical and fat stores present at birth are less important for neonate survival. Thus, in low-quality environments, female poeciliids should sacrifice offspring number for quality, whereas in high-quality environments, offspring number is boosted at some cost to quality. Reznick et al. (1996) simulated poor and good environmental conditions in the laboratory with low- and high-food treatments. They found that two species of lecithotrophic poeciliids produced fewer, heavier offspring when raised on the low-food level than when raised on the high-food level. The increased mass was primarily fat. However, a matrotrophic species that produced very small ova gave birth to large offspring on high food, and to smaller ones on low food, inconsistent with their model. In addition, offspring size and number of the matrotrophic species closely tracked changing food levels over multiple broods; both increased when food level was increased. For the lecithotrophic species, the characteristics of offspring changed more slowly with changing food level. Reznick et al. (1996) suggested that matrotrophy acted as a nonadaptive constraint in this case. They proposed that matrotrophy limited the ability of females to adapt to low-food conditions through increasing offspring size, because reproduction was largely or solely fueled by recently acquired energy (Fig. 1).

Sailfin mollies differ from the three species studied by Reznick and his colleagues in several ways, including their generally larger size as adults and their

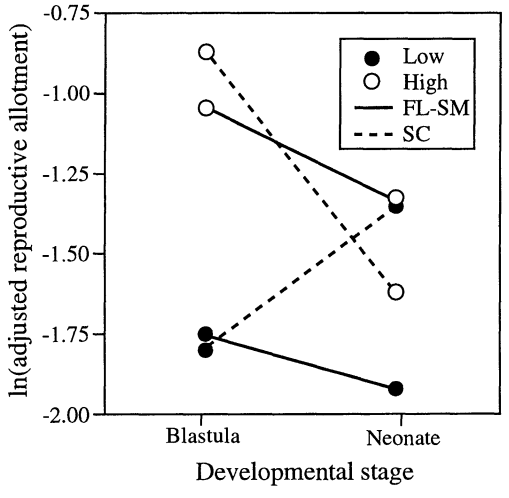

FIG. 5. Reproductive allotment (%) adjusted to the grand mean brood size, plotted relative to the developmental stage for results from experiment 2. Source population codes are as in Fig. 3.

TABLE 5. Analysis of reproductive allotment (percentage of female mass) at the blastula and neonate developmental stages under the two feeding treatments of experiment 2. Source refers to the source population. Adjusted means were estimated at the log-transformed grand mean female mass of −1.099 (untransformed 333.0 mg). Only the significant model terms are reported.

A) ANOVA of reproductive allotment

Source	SS	df	MS	*F*	*P*	CD
Treatment	1.938	1	1.938	5.981	0.017	5.0
Female dry mass	2.200	1	2.200	6.789	0.011	5.7
Treatment × Stage	3.460	1	3.460	10.676	0.002	8.9
Source × Stage	1.620	1	1.620	4.999	0.028	4.2
Stage × Mass	1.367	1	1.367	4.219	0.043	3.5
Source × Stage × Mass	1.681	1	1.681	5.188	0.025	4.3
Error	27.223	83	0.324			

B) Adjusted mean reproductive allotment

			Adjusted means		
Food level	Source†	Stage	Transformed (±1 SE)	Untransformed (×100)	*N*
Low	FL-SM	blastula	−1.767 (0.150)	17.1	17
Low	FL-SM	neonate	−1.934 (0.238)	14.5	7
Low	SC	blastula	−1.821 (0.228)	16.2	7
Low	SC	neonate	−1.361 (0.296)	25.6	4
High	FL-SM	blastula	−1.039 (0.155)	35.4	15
High	FL-SM	neonate	−1.339 (0.225)	26.2	8
High	SC	blastula	−0.862 (0.144)	42.2	18
High	SC	neonate	−1.635 (0.187)	19.5	9

† Source population codes are as in Table 2.

production of more and larger offspring. The experiments reported here indicate little response of sailfin molly offspring size to female scope for reproduction; the trend in both experiments, not statistically significant in either case, was for high-food females to produce slightly larger offspring after adjusting for brood size. Offspring fat was not estimated in the first experiment, but in the second experiment there was no significant difference in the fat content of offspring from the two feeding treatments; if anything, the offspring of high-food females tended to be fatter. Sailfin mollies adjusted egg size rather than offspring size in response to changing conditions.

Can the findings for mollies be explained by the model of Reznick and colleagues? Their model assumes that offspring fitness is more dependent on offspring size in low-quality environments than in high-quality ones. Field studies with mollies indicate that offspring size may be more closely tied to fitness in some years and some environments than in others (Trexler et al. 1992). Additionally, their model assumes that a female's environment can be used to predict that of her offspring. This may be true for the tropical species studied by Reznick et al. (1996), but sailfin mollies inhabit a wide range of environments that are often quite variable in physical conditions such as salinity. Our field studies indicate that molly life tables can vary greatly among years in the same location (Trexler et

TABLE 6. Analysis of percentage of fat of blastulae and neonates produced by females raised under two feeding levels in experiment 2. Adjusted means were compared at the log-transformed grand mean brood size of 3.455 offspring (untransformed 32).

A) ANOVA of percentage fat

Source	SS	df	MS	*F*	*P*	CD
Source population	73.640	1	73.640	3.003	0.087	
Source × Developmental stage	146.445	1	146.445	5.973	0.017	4.3
Stage × Brood size	1172.609	1	1172.609	47.826	<0.001	34.8
Error	1912.418	78	24.518			

B) Adjusted mean percentage fat

Source population†	Stage	Adjusted mean fat (%) ± 1 SE	*N*
FL-SM	blastula	9.415 (0.809)	33
FL-SM	neonate	10.011 (1.230)	12
SC	blastula	14.358 (0.929)	23
SC	neonate	9.167 (1.140)	14

† Source population codes are as in Table 2.

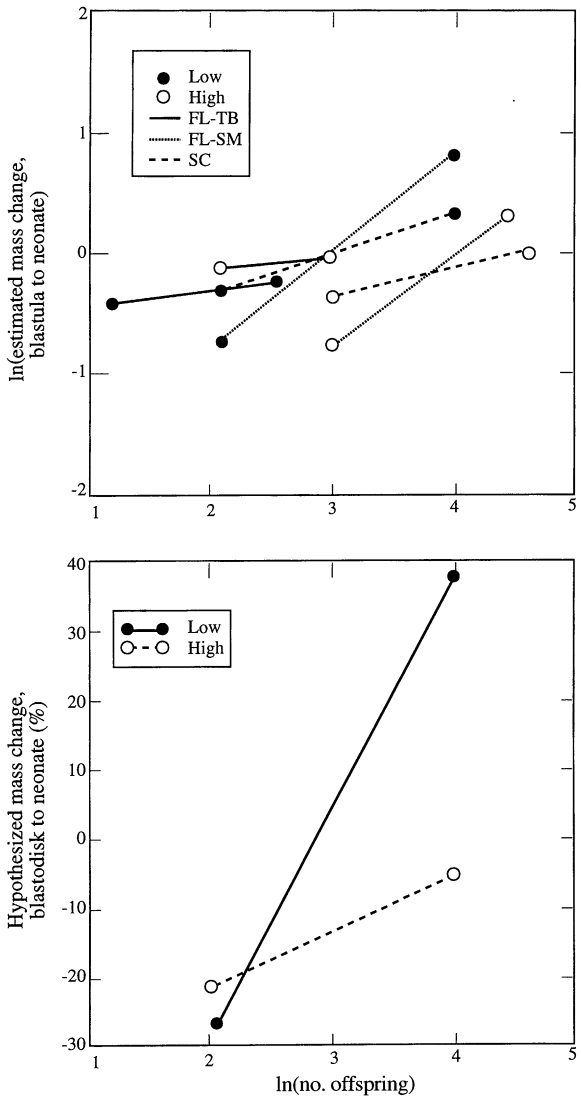

FIG. 6. An index of matrotrophy, relative to brood size, for females raised in both experiments. The top panel shows the estimated mass change of embryos during development for fish from both experiments. These estimates were obtained by separately regressing the mass of blastulae and neonates on brood size and subtracting their respective predicted values. FL-TB (Tampa Bay, Florida) results are from experiment 1 and FL-SM and SC results are from experiment 2. Line length indicates the range of brood sizes observed. The bottom panel shows the hypothesized percentage change in dry mass from fertilization to parturition for sailfin mollies. This hypothesis was obtained by repeating the regression analysis used for the top panel on data pooled from both experiments, and by adjusting the results to a percentage change.

al. 1992), as do conditions affecting growth and development (Trexler and Travis 1990). This unpredictability may favor females that maximize offspring size, or at least that produce offspring of a minimum size in all broods.

Charnov et al. (1995) suggest that female poeciliids with small brood sizes may invest more energy in each offspring than do those with larger broods, if there is a trade-off of offspring size and number. Female sailfin mollies with relatively small broods invested more per *offspring* initially, via lecithotrophy, than did those with larger broods. However, if matrotrophy is energetically more expensive than lecithotrophy, they may invest less per *neonate*. There appears to be an ovum size–ovum number trade-off in mollies that may be diminished by production of small ova nourished by matrotrophy. Ovum size may place greater limitation on brood size than neonate size.

My results failed to support a bet-hedging model for brood reduction, because the least brood reduction was observed in a low-food treatment. The bet-hedging model predicts greatest brood reduction in poor-quality environments (Kozlowski and Stearns 1989). Although aspects of my results are hampered by small sample size, the high-food treatment group from SC had the greatest gestational decline in brood size (39.9%) with moderate sample sizes (18 broods at the blastulae stage and 11 at the neonate stage). The selective-abortion hypothesis remains viable. This could be tested by examination of size-adjusted fecundity from females exhibiting varying levels of inbreeding (D. Reznick, *personal communication*). The selective-abortion model does not explain the low level of brood reduction estimated for the SC low-food group, but it could explain the substantial and similar amount of brood reduction observed in the three other treatment groups. Both hypotheses warrant further examination in poeciliid fishes.

Why aren't female sailfin mollies matrotrophic all of the time? Reznick et al. (1996) suggested that obligate matrotrophy may yield suboptimal solutions to the offspring size–number trade-off by fueling reproduction largely or solely by recently acquired energy. Facultative matrotrophy may permit maximum flexibility in the use of stored and recently acquired energy, and may provide a solution to limitations from strict matrotrophy. Also, matrotrophy may be more energetically expensive per neonate than lecithotrophy, although the investment is spread over gestation (Fig. 1). Thus, facultative matrotrophy may be an adaptation to increase brood size while diminishing the trade-off of offspring size and brood size seen in obligate lecithotrophic poeciliid species. Experiments like those of Reznick et al. (1996), or using labeled food resources, are needed to trace the roles of recently acquired and stored energy in reproduction and the significance of facultative matrotrophy in resource investment.

ACKNOWLEDGMENTS

Thanks to Joseph Travis, who supported my research for many years, Sandra Blake, who helped with experiment 1, and David Reznick, for discussions of poeciliid reproductive biology. Jody Haynes, Joe Travis, Andy Turner, and Tom Turner provided helpful comments on drafts of this paper. Various aspects of this project were supported by faculty development grants from Eckerd College and the University of Mississippi to the author, and NSF grant BSR 88-18001 to J. Travis.

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