Copeia, 1995(1), pp. 97–104

Lipid Dynamics and Growth Relative to Resource Level in Juvenile Eastern Mosquitofish (*Gambusia holbrooki*: Poeciliidae)

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We examined early energy allocation patterns of eastern mosquitofish (*Gambusia holbrooki*) reared on three dietary regimes by measuring growth and lipid storage at several points during the first 40 days of life. A high food treatment produced growth increments similar to those previously found in natural mosquitofish populations, whereas intermediate (one-half the quantity of high) and low (one-quarter the quantity of high) food treatments produced significantly lower growth increments. Lipid content was about 20% of dry mass at birth and declined for the first five days of life in all three treatments. After this initial period of lipid use, lipid levels increased asymptotically in all treatments. Lipid content was positively correlated with feeding level and plateaued at significantly different levels for each treatment. These three laboratory-reared groups had significantly higher percent somatic lipids than two populations of wild fish of similar size, probably because of differences in food and activity levels between laboratory and field environments. Size at birth was positively correlated with size at two weeks of age but was not significantly correlated with size beyond 15 days of age. Birth size was uncorrelated with subsequent growth or lipid content. No trade-offs between growth and lipid storage were found at any level examined in this study. We suggest that differences in energy acquisition or metabolic efficiency may swamp differences in resource allocation between these two energy compartments.

ENERGY allocation patterns in animals should be under selection to optimize the competing functions of metabolic maintenance, growth, and reproduction, as well as storage for future use in the first three activities (Gadgil and Bossert, 1970; Caswell, 1983; Winkler and Wallin, 1987). Trade-offs should exist between these energy compartments; energy metabolized for maintenance, for example, cannot be used for reproduction. The pattern of allocation is interesting from both physiological and evolutionary perspectives. Selection should act to maximize lifetime reproductive success (Williams, 1966), and that success is partly determined by physiological mechanisms that control energy partitioning.

One aspect of an individual's energy budget, storage, is especially interesting. Storage in the form of lipids, primarily triglycerides and free fatty acids, allows energy procured at one time to be used at another time (Derickson, 1976; Iles, 1984; Reznick and Braun, 1987), thus circumventing immediate allocation "decisions" among maintenance, growth, and reproduction. When energy is abundant, it may be captured in excess and saved for periods of lower resource abundance or higher energetic demands, such as reproduction.

Livebearing fishes of the family Poeciliidae have been used in several studies of energy storage. The western and eastern mosquitofishes (*Gambusia affinis* and *G. holbrooki*, respectively) and the sailfin molly (*Poecilia latipinna*) have all been studied with respect to storage and later use of lipids for growth and reproduction (Reznick and Braun, 1987; Meffe and Snelson, 1993a, 1993b). In all cases, reproductive adults were used, and the general pattern discerned was increased lipid storage during nonreproductive periods (late summer/fall) and lipid use (depletion) during overwintering and especially during spring reproduction. Lipid storage apparently plays an important role in poeciliid life histories by providing an energy source for periods of high demand but low resource availability.

To date, most life-history studies on energy allocation patterns in poeciliids have been on reproductive adults (Reznick and Braun, 1987; Meffe, 1991; Weeks, 1993). Although these fish are born with a large store of lipids and have lipid stores at maturity that are used in reproduction, the dynamics of lipid storage and use between birth and maturity are unknown. This study addresses that period of the life history. We conducted a laboratory study of juvenile

TABLE 1. FEEDING SCHEDULE FOR MOSQUITOFISH IN THE THREE TREATMENT GROUPS. Brine shrimp nauplii were delivered live to each container volumetrically. Each ml of solution delivered approximately 0.35 ± 0.14 mg dry mass of nauplii.

Age (days)	Treatment		
	Low (ml)	Medium (ml)	High (ml)
1–5	1	2	4
6–16	2	4	8
17–26	3	6	12
27–40	4	8	16

eastern mosquitofish to determine lipid storage relative to growth patterns from birth to 40 days of age.

MATERIALS AND METHODS

The eastern mosquitofish (*Gambusia holbrooki*, hereafter "mosquitofish") is a common species of lakes, ponds, marshes, and riverine backwaters of the east and Gulf coasts of the United States. Along with the western mosquitofish, it has been introduced throughout much of the world under the mistaken notion that it effectively controls mosquitos (Courtenay and Meffe, 1989). The fish grows to 2–5 cm and probably lives one year or less in most habitats. It is internally fertilized and livebearing, producing several broods per year, each of up to several dozen neonates. It is especially good for studying energy allocation because of its well-documented reproductive patterns and distinct periods of pregnancy (Constantz, 1989; Reznick and Miles, 1989).

We used mosquitofish from a former nuclear cooling reservoir, Pond C, on the United States Department of Energy's Savannah River Site near Aiken in west-central South Carolina. This population previously has been studied with respect to various life-history characteristics (Meffe 1990, 1991, 1992). Pregnant females were captured in the field and taken to the laboratory in June 1992. Fifteen offspring from each of 10 broods collected over a four-day period were used in an experiment designed to determine patterns of lipid storage and growth during the juvenile period.

Experimental procedure.—We reared juveniles at three food levels and measured body size and lipid content at five periods of development: ages 5, 10, 15, 25, and 40 days. We also measured lipids in extra brood members at birth. In this way, we could determine growth and stored lipid content in juveniles reared on different food levels. Because lipid analysis involves destructive sampling, each fish was used for lipid measurement only once, at a preselected day. Thus, this is a cohort study; temporal patterns of lipid storage were determined across a cohort, rather than in the same individuals over time.

Lengths of all experimental fish were measured at birth using a computer image analysis system with MorphoSys® software. Fish were never directly handled for measurement. Their dorsal images were captured by a video camera and measured on MorphoSys® to the nearest 0.01mm. Three standard length (SL) measurements were taken on each fish, and their mean value was used.

The 150 fish (10 broods, 15 per brood) were raised individually in 475-ml plastic cups arrayed on a table. Fish were reared on one of three diets (Table 1), representing low, medium, and high food availability. The medium diet was twice the quantity of the low diet, and the high diet was four times the low. Food consisted of brine shrimp nauplii, hatched out daily, and was applied in controlled volumetric quantities using an adjustable pipette. Amounts for the three treatment groups were incrementally increased with age (Table 1).

All cups were cleaned, and water was changed every three to five days. Temperatures were not directly controlled, but the array was spatially homogeneous on a 1.5 × 2.5 m table. Temperatures across the array were measured daily and indicated no consistent thermal differences. Even if there were thermal differences, the experiment was laid out so that each brood and treatment was equally represented in rows and columns.

On each of five preselected days, 10 randomly selected fish (one from each brood) in each food treatment group (30 fish total) were killed, before feeding, in 10% formalin and preserved in 5% buffered formalin for lipid measurement. Lipid analysis was conducted within one month of preservation.

Lipid analysis.—None of the experimental fish had sexually matured by the end of the experiment; therefore, all estimates reflect somatic lipid content only. Standard lengths of preserved fish were measured as above. Fish were then diced into small pieces and placed in tared, 2.5-ml glass vials. Vials and contents were placed in a drying oven for two days at 55 C, placed in a desiccating chamber at 0% humidity for another two days, and then transferred to a glove box at 0% humidity for one day, where

they were weighed to the nearest 0.01 mg on a Mettler AE240 electronic digital balance. This, minus the tare mass, provides the total dry mass of the fish.

Lipids were extracted by adding approximately 2 ml petroleum ether to each vial, covering the vials for 45–60 min, removing the ether, and repeating for a total of six ether washes. Preliminary studies indicated that six washes were in excess of the amount needed to remove all measurable storage lipids. After washing, samples were dried and reweighed as above. The difference in pre- and postextraction mass is the amount of storage lipid contained in the individual; petroleum ether removes mostly nonpolar (storage) lipids, leaving polar (structural) lipids largely intact (Dobush et al., 1985).

The remaining sibling neonates (n = 3–22) from each of the 10 clutches were preserved at birth. Because of their small size, these fish were combined by clutch to measure average lipid content at birth for each clutch and analyzed for lipids in the same manner described above.

To compare lipid storage of laboratory-reared individuals with wild fish, samples of juveniles were collected from Pond C, the source population for the experiment, plus Risher Pond, a nearby pond population that previously had been studied. Fish were preserved in the field in 5% buffered formalin and underwent similar lipid extractions.

Statistical methods.—Data were analyzed using a randomized-block, factorial analysis comparing growth and lipid storage in a 3 (feeding treatments) × 5 (age periods) ANOVA using the 10 females as blocks. The female × feeding and female × age interaction terms were not significant in preliminary analyses ($P > 0.15$) and were not included in the final model. Therefore, the treatment main effects were tested against the error mean square in both analyses.

Data were analyzed with the General Linear Models, Univariate, and Correlation procedures of the Statistical Analysis Systems statistical package (SAS Institute, Inc., 1985). Growth was estimated by measuring SL of a fish at the end of the experiment and subtracting from that its SL at birth. Dry mass was tightly correlated with SL ($R^2 = 0.88, P < 0.001$). To avoid redundancy, we only report analyses of growth in terms of SL. Lipid content is reported as the percentage of total dry mass found to be lipids. Both dependent variables were normally distributed. Correlations among length at birth, growth increment, length at end of experiment, and lipid content were analyzed by considering individuals within each age × feeding treatment.

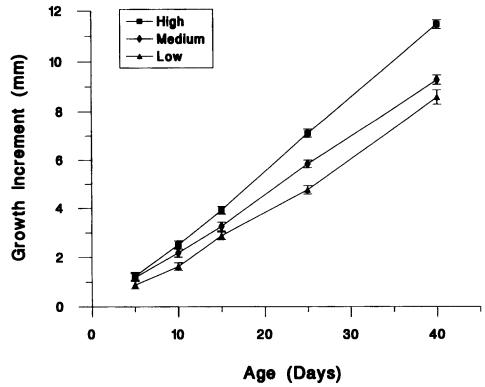

Fig. 1. Means ± 1 SE for growth in length for the three feeding treatments over the five age groups. Growth is defined as the change in standard length from birth to age x.

Percent lipids in experimental fish was compared to that in wild-caught fish using an analysis of covariance, with standard length as the covariate. To compare a size range within each feeding treatment to the wild-caught fish, juveniles were pooled across the 10 females and five age treatments. The relationship of standard length to percent lipids was homogeneous among treatments, as assumed in the ANCOVA model.

Results

Growth was linearly related to age in all three treatments (Fig. 1; $F_{4,105} = 968.69, P < 0.0001$), and was significantly different among feeding treatments ($F_{2,105} = 92.17, P < 0.0001$), with High > Medium > Low food [Ryan-Einot-Gabriel-Welsch Multiple Range Test (SAS Institute, Inc., 1985); $P < 0.05$]. The ANOVA indicates that there was a feeding treatment × age interaction ($F_{8,105} = 10.40, P < 0.0001$), which is evidenced by a steeper slope of growth with age for the high-food group (Fig. 1). Offspring from different females also grew at different rates ($F_{9,105} = 2.41, P < 0.05$).

The percent of somatic lipids increased over time (Fig. 2; $F_{4,105} = 6.92, P < 0.0001$), but the increase was asymptotic over the 40-day period studied. Fish in the three feeding treatments had significantly different levels of lipid stores ($F_{2,105} = 27.63, P < 0.0001$). Fish in the low food treatment plateaued at 13.5% lipids, which was reached at 15 days of age. Fish in both the high and medium treatments continued to increase in percent lipids but at a slower rate by

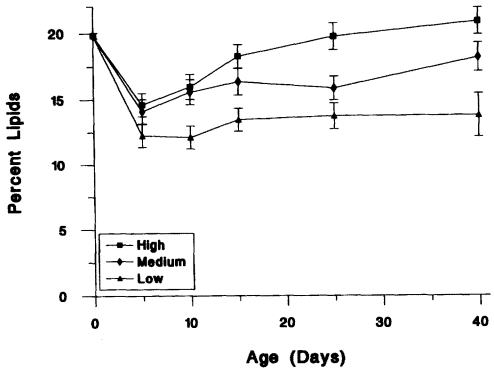

Fig. 2. Means ± 1 SE for the percent of total dry mass found to be resulting from lipids for fish in the three feeding treatments over the five age groups. Percent lipids at birth is shown at age-zero for 100 sibling offspring of the 150 experimental fish.

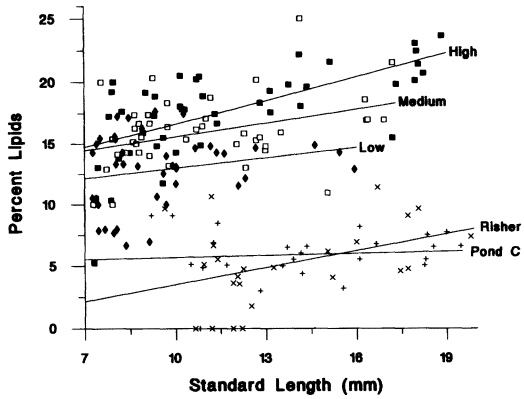

Fig. 3. Relationship of percent lipids to standard length for mosquitofish in all three feeding treatments and for two natural *Gambusia holbrooki* populations. Symbols are as follows: high food = solid squares, medium food = open squares, low food = solid diamonds, Risher Pond = X, and Pond C = +. Lines show the least-squares regression of percent lipids on standard length for all five treatments. Regression equations are as follows: High: $y = 10.31 + 0.63x$, $n = 45$, $r = 0.636$, $P < 0.01$; Medium: $y = 11.85 + 0.37x$, $n = 43$, $r = 0.363$, $P < 0.05$; Low: $y = 10.20 + 0.28x$, $n = 41$, $r = 0.207$, $P > 0.05$; Risher Pond: $y = -1.03 + 0.46x$, $n = 24$, $r = 0.392$, $P < 0.05$; Pond C: $y = 5.18 + 0.05x$, $n = 25$, $r = 0.084$, $P > 0.05$.

40 days (Fig. 2). The analysis of variance showed no significant feeding treatment × age interaction ($F_{8,105} = 1.05$, $P > 0.40$), nor any significant female effects ($F_{9,105} = 1.66$, $P > 0.10$). The average percent lipids at birth for 100 siblings of the 150 experimental fish is shown at age zero (Fig. 2) but was not included in the ANOVA. Clearly, newborn Gambusia rely on lipid stores in the first few days of life, which can be seen by the decline in the percent lipids from 20% to between 15 and 12% in the first five days of the experiment (Fig. 2).

The relationship of percent lipids in somatic tissue to standard length was compared among experimental treatments and to that in wild caught fish of similar size. There were no apparent trade-offs between growth and lipid storage; such a trade-off would have been evidenced by a negative correlation between size and lipid storage within each feeding level of the experiment. Standard length and somatic lipid content were positively correlated for fish within all three experimental treatments, which was mirrored in both the Risher Pond and Pond C wild-caught fish (Fig. 3). Also, lipid content among treatments was positively correlated with food ration even when correcting for differences in size ($F_{4,172} = 134.16$, $P < 0.0001$). Pairwise comparisons of size-corrected lipid content revealed that fish in all three laboratory treatments had higher percent lipids than fish of equivalent standard length from either field location.

Birth size was significantly positively correlated with final size in six out of nine cases in the first 15 days of growth, indicating that initial size differences persisted for the first two weeks of juvenile growth (Table 2). This correlation decreased as the juveniles aged. Birth size was not significantly correlated with growth increment in any group, but 11 out of 15 comparisons were negative, indicating a trend toward larger fish at birth growing more slowly. Neither birth size, final size, nor growth were consistently correlated with lipid content, the latter two again reflecting the lack of evidence for a trade-off of growth and lipid storage. Predictably, growth and final size were positively correlated in all comparisons and significantly so in eight cases.

Discussion

We addressed the effects of resource level on early mosquitofish life history by measuring juvenile lipid storage and growth increment under three feeding regimes. Our intent was to produce "normal" and resource-limited conditions by designating different food levels to the three treatments a priori. This was done by using experience to select a food level that would be likely to result in normal growth and then designating fractions of that diet for resource-limited treatments. The feeding regimes we chose did, in fact, produce a statistically significant treatment effect on growth and lipid stor-

TABLE 2. PEARSON CORRELATION COEFFICIENTS FOR STARTING SIZE, GROWTH, ENDING SIZE, AND PERCENT LIPIDS IN JUVENILE MOSQUITOFISH IN EACH FEEDING BY AGE TREATMENT GROUP. Sample sizes for each treatment combination are given in the column labeled N. The units for age are days.

Feeding	Age	n	Birth size/ end size	Birth size/ growth	Birth size/ % lipids	Growth/ end size	Growth/ % lipids	End size/ % lipids
Low	5	10	0.803**	−0.206	0.172	0.418	−0.449	−0.113
	10	10	0.599	−0.170	−0.308	0.687*	0.485	0.167
	15	10	0.383	−0.545	−0.124	0.566	0.271	0.177
	25	8	0.303	−0.025	−0.588	0.945***	0.239	0.036
	40	3	−0.601	−0.715	0.257	0.988	−0.859	−0.927
Medium	5	9	0.701*	−0.308	0.395	0.462	0.063	0.415
	10	9	0.675*	0.012	0.514	0.746*	0.407	0.643
	15	8	0.719*	0.179	0.273	0.812*	−0.648	−0.296
	25	10	0.412	−0.174	0.135	0.825**	−0.395	−0.289
	40	7	0.390	0.097	−0.294	0.954***	−0.043	−0.128
High	5	10	0.757*	−0.336	0.149	0.361	0.542	0.523
	10	9	0.770*	−0.305	−0.463	0.373	0.320	−0.237
	15	10	0.547	−0.306	−0.133	0.630	−0.246	−0.325
	25	8	0.572	0.099	0.311	0.873**	0.603	0.650
	40	8	0.309	−0.106	0.506	0.913**	0.612	0.793*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

age, with decreased resources reducing both growth and percentage of lipids in juvenile fish. Our three food treatments resulted in growth increments near and below those found in the wild population in Pond C. Meffe (unpubl. data) found that the first annual brood of mosquitofish in the spring of 1990 in Pond C grew 8.2 mm in 25 days. The high food group in our experiment grew 7.1 mm in 25 days, whereas the medium and low food groups grew 5.8 and 4.8 mm in that period, respectively. As reflected by growth increments, our laboratory results represent allocation patterns under two levels of resource limitation (medium and low food treatments), and a near-normal level of resource availability (high food treatment).

In all three treatments, lipid levels were high at birth, declined for the first 5–10 days, and then increased asymptotically in all treatments. In the 40 days of the experiment, only the high food treatment group achieved a lipid level equivalent to that measured at birth. Similar patterns of lipid use have been reported for other fish species. In larval plaice (*Pleuronectes platessa*), triglyceride concentration decreased from 7.3% to a low of 4.6% of total body mass during the first 15 days after hatching (Ehrlich, 1974). After this period, triglyceride levels increased for two weeks, plateaued at 7.8%, and after 40 days of age again declined (Ehrlich, 1974). Similarly, in Atlantic herring (*Clupea harengus*), triacylglycerol content declined for the first 10 days of development and then increased thereafter (Fraser et al., 1987). Losses of 30–50% of total lipids have been reported for cod (*Gadus morhua*) during the first two weeks of life (Fraser et al., 1988), and lipid metabolism (and thus loss) in the striped bass (*Morone saxatilis*) was recorded for the first 25 days of larval development (Eldridge et al., 1982). These patterns of initial lipid use followed by later lipid deposition in early larval life may indicate lack of feeding in the earliest larval stages. Initial lipid content may determine how long these stores last (Blaxter and Hempel, 1963), so levels of parental investment may be crucial to this period of juvenile survival.

In the current experiment, mean lipid levels ranged from 12–21% of total somatic dry weight, which is consistent with findings in other fish species. Fraser et al. (1988) reported a range of 14.2–21.2% of dry mass for the first two weeks after hatching in cod larvae. Our results also correspond well with reports of lipids in herring aged 1–14 days, which had levels from 10.0–23.7% (Tocher et al., 1985). Similarly, the minimum level recorded at five days of age for our low food treatment (12% of dry mass) fell within the range of values, 6–18%, reported by Reznick and Braun (1987) for immature *G. affinis* in natural populations. By age 40 days, our high-food fish had attained a lipid level of approximately 21% of total body weight, slightly exceeding the maximum of 20% observed for *G. affinis* by Reznick and Braun (1987).

Though our results correspond well with those of other laboratory studies, the lipid levels were significantly higher than those measured from

similarly sized individuals from the field. *Gambusia holbrooki* collected from two natural populations on the Savannah River Site, including the pond from which the parents of our experimental fish were collected, contained significantly lower lipid levels than measured in the laboratory. Higher levels of stored lipids in laboratory-reared relative to wild-caught fish have been reported in several studies (Love, 1970; Balboutin et al., 1973; Ehrlich, 1975) but have not been attributed to particular effects. Certainly there are obvious differences between field- and laboratory-reared fish. Diets are different, both quantitatively and qualitatively. Fish in the laboratory are more sedentary than those in the field and do not have to search for food or escape from predators, an apparent cause for higher percentage of body fat in laboratory-reared fish. Fraser et al. (1988) found that cod larvae (*Gadus morhua*) in containers with live prey depleted lipid stores at a quicker rate than larvae fed nonliving prey, presumably because of the increased foraging activity necessary to consume live prey. Ehrlich (1975) found that prefeeding herring (*Clupea harengus*) and plaice (*Pleuronectes platessa*) larvae reared in the laboratory had higher fat stores than wild-caught individuals of equivalent stage and concluded, "activity or other behavioral differences may play a regulatory role in deposition of fat stores." This overestimate of lipid content may prove problematic for extrapolating results of energy partitioning studies in laboratory-reared fish to conditions encountered in the field.

Both birth size and initial lipid content, which are controlled by parental investment, may have serious ramifications for fitness in terms of competitive ability, growth rate, survivorship under resource deprivation, and susceptibility to predation (Blaxter and Hempel, 1963; Salthe, 1968; Kaplan, 1980). Reznick (1981) found birth size to be a function of maternal genotype for *G. affinis*, and significant differences in birth size have been documented among full-sib families in *G. holbrooki* (SCW and GKM, unpubl. data). Our present results indicate that the correlation of birth size with juvenile size diminished over the first two weeks of life and that birth size was not significantly correlated with growth increment or lipid content. Similar patterns of diminishing differences in body size have been observed for rainbow trout and herring; however, birth size was significantly correlated with growth rate in these cases (Blaxter and Hempel 1963; Gall 1974). Thus, if the current results typify conditions in the field, differential parental investment in *G. holbrooki* must impart greater fitness in the first two weeks of life if it is to have any effect. Birth size has been shown to significantly affect survival in the first four weeks of life in another poeciliid fish, *Heterandria formosa* (Henrich, 1988); therefore, we might expect similar effects in *Gambusia*.

There was no apparent trade-off between growth and lipid storage at any level of comparison in this study. Any organism faced with limiting resources required by many processes must necessarily decrease allocation to one process if it is to simultaneously increase allocation to another (Levins, 1968; Bell and Koufopanou, 1986; Stearns, 1989). Because both growth and storage require energy from food, a trade-off is expected if food is limiting (Bell and Koufopanou, 1986). Such a trade-off should produce a negative correlation between these two processes. However, our results indicate a positive correlation between storage and growth for laboratory-reared fish, even the food-limited individuals, and between storage and size for wild-caught fish. One reason that a trade-off may not be observed is that individuals may differ in their ability to obtain energy. Van Noordwijk and deJong (1986) indicate that the sign of the correlation between two competing, independent processes is determined by the relative variances in acquisition and allocation of resources. They state that differences in efficiency of resource gathering can swamp differences in resource allocation patterns. This would result in a positive correlation among growth, reproduction, and lipid storage. We cannot test this hypothesis in our experiment because of our inability to quantify inherent differences in energy acquisition and metabolism among individuals. Therefore, exploration of the observed positive correlation between growth and lipid storage needs to be left to detailed physiological experiments that can specifically measure differences in ingestion rates, digestive efficiency, or metabolic rates.

Our study indicates that juvenile growth and lipid storage patterns are, not surprisingly, affected by resource level. But these patterns are also affected by metabolism of lipid stores in the first days of life that takes several weeks to restore; this postpartum lipid use may in turn be affected by the amount of lipid stores remaining after embryonic development, which in turn may be a function of initial ovum size. These details are inaccessible in our present study. We found, additionally, that there is no discernible trade-off between growth increment and lipid storage in juvenile mosquitofish; a fast-growing individual is not likely to store less lipids than a slow-growing individual. Thus, lipid storage is not a simple process by which excess energy is merely

dumped into fat, and there is apparently not a simple decision to be made between growth and lipid storage. The dynamics of these energetic pathways are more complicated; an individual especially good at gathering energy, or particularly efficient metabolically, may be good at both growth and storage, compared to other individuals. The more interesting question is why there is not strong selection against those who grow slowly and store lipids poorly. We speculate that those particular phenotypes are at a selective advantage in other environments or that these phenotypic differences are environmentally induced rather than heritable, thus maintaining the apparently disadvantageous variation in juvenile development.

Acknowledgments

We thank F. R. Hensley and J. D. Congdon for critical reviews of the manuscript and D. Fletcher, S. D. Wilkins, T. M. Farrell, and P. G. May for assistance in the laboratory. This study was funded by contract DE-AC90-76SROO-819 between the United States Department of Energy and the University of Georgia, and STH was supported by the SREL Undergraduate Research Participation Program.

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