

THE EFFECT OF FOOD LIMITATION ON LIPID LEVELS, GROWTH, AND REPRODUCTION IN THE MARBLED SALAMANDER, *AMBYSTOMA OPACUM*

DAVID E. SCOTT¹ AND MICHELE R. FORE^{1,2}

¹*Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802, USA*

ABSTRACT: Assimilated food energy is partitioned into four compartments: maintenance, growth, reproduction, and stored energy in the form of lipids. Food availability is limited in many natural systems, so organisms face trade-offs in allocating energy to these competing compartments. We conducted a dietary study with female marbled salamanders, *Ambystoma opacum*, to examine the relationship between food level and energy allocation patterns. Feeding treatments produced differences in total lipid amounts, growth rates, and reproductive traits. Females in the high-food treatment group exhibited larger body size, higher lipid levels, greater proportions of total lipids, and larger clutch size than low- and medium-food animals. In addition, 60% of the high-food females were reproductive at the end of the experiment, compared to 42% of the females sustained at medium-food levels and 12% on the low-food regimen. After removal of an apparent outlier from analyses, a female's mean egg size and egg lipid investment per egg depended on food level through its effect on body size; larger females tended to have bigger eggs. Collectively these results suggest that food availability affects traits related to individual fitness and may also influence the population dynamics of a species.

Key words: *Ambystoma opacum*; Egg size; Fecundity; Food stress; Growth; Lipids; Reproduction; Reproductive frequency; Resource allocation; Salamander

REPRODUCTIVE characteristics such as age at maturity, clutch size, and reproductive frequency are important life-history traits that influence both individual

fitness and population dynamics. Numerous recent studies have documented substantial plasticity in reproductive traits (Bernardo, 1993; Etter, 1989; Ford and Seigel, 1989, 1994; Kaplan, 1987; Maiorana, 1976; Reznick, 1983, 1990; Reznick and Yang, 1993). Knowledge of which traits are plastic, and the factors that influence this phenotypic plasticity, is critical to un-

² PRESENT ADDRESS: Aquatic Ecology Laboratory, The Ohio State University, Columbus, OH 43212, USA.

derstanding the relationships among phenotype, fitness, and selection.

If food resources in natural systems are limited, then trade-offs will occur in energy allocated among the competing processes of maintenance, growth, energy storage, and reproduction (Congdon et al., 1982; Dunham, 1978; Dunham et al., 1989; Fitzpatrick, 1976). Thus, resource availability is one environmental factor that may strongly affect reproductive traits. Condition (i.e., nutritional state) may influence reproductive output (Fraser, 1980) and/or reproductive frequency (Martin, 1977). An individual faced with limited food availability must make "decisions" concerning whether to reproduce or not, and (if so) how much total energy to allocate to reproduction. In many ectotherms, total clutch mass is positively related to body size (Berven, 1988; Ford and Seigel, 1989; Kaplan and Salthe, 1979; Salthe, 1969; Salthe and Mecham, 1974; Semlitsch, 1985; Wilbur, 1977). Clutch size/body size relationships may vary in relation to changes in food availability (Ballinger, 1977; Reznick and Sexton, 1986) and feeding opportunity (Bruce, 1969).

Variation in environmental factors such as food resources also affects the "packaging" of reproductive allocation into individual offspring (Dunham et al., 1989). In salamanders, size-specific maximum clutch mass may be constrained by the volume of the body cavity (Kaplan and Salthe, 1979). Consequently, at maximum clutch volume, a trade-off between egg number and egg size may occur. However, there is evidence that females in some salamander populations do not maximize their reproductive output (i.e., clutch volume) every breeding season (Maiorana, 1976); individuals do not always make the largest clutch possible within the constraints of body size (Fraser, 1980).

In the marbled salamander, *Ambystoma opacum*, a significant positive correlation between clutch size and body size is generally observed. However, the amount of variation in clutch size explained by body size has a wide range; in populations of *A. opacum* studied by Kaplan and Salthe (1979), from 7-77% of the

variation in clutch size was explained by body size. Similar variability has been observed in numerous other species of salamanders (e.g., Anderson, 1960; Bruce, 1969; Fitzpatrick, 1973; Houck, 1977; Tillely, 1968). In one natural population of *A. opacum* studied during one year, body size explained 79% of the variation in clutch size (Scott, 1994), and a trade-off between egg size and egg number was observed (see Results), which may indicate that females were at maximum clutch volume in that year. Much of the unexplained variation in size-specific clutch size may reflect variation in nutrition (Fraser, 1980; Maiorana, 1976). In addition, food availability may influence reproductive frequency (Ballinger, 1977; Maiorana, 1974), as the initiation of the next ovarian cycle following reproduction is influenced by food level (Jørgensen, 1982). Because prey availability may be the most important environmental factor that induces plasticity in reproductive traits, our purpose in this study was to quantify the effects of food level on energy allocation in females of *Ambystoma opacum*. Experimental manipulation of food levels provided a controlled means of exploring the causal basis of observed field patterns.

METHODS

During October 1988, we captured approximately 50 pairs of male and female *A. opacum* during a breeding migration to Squirrel Bay, a Carolina bay on the U.S. Department of Energy's Savannah River Site in Aiken County, South Carolina. We placed the animals in two 2.5-m diameter cattle tanks with soil and vegetative cover, where they courted and females nested and oviposited. We collected 46 females from the cattle tanks on 15 February 1989 for the dietary study; remaining animals were released at the site. Experimental animals were held in a cattle tank for an additional 5 wk without feeding. Previous studies of post-breeding, wild-caught females indicated that they do not feed during the breeding season and leave the breeding site with substantially reduced lipid stores (Kaplan and Crump, 1978; D. E. Scott, unpublished data). Thus, at the

beginning of the experiment, all females had bred the previous year and all presumably had reduced energy stores. During the last week in March, we brought the animals to the laboratory and housed them in individual containers in a single environmental chamber on a 12:12 h daily light and temperature cycle. The daily temperature extremes (21:15 C) were determined from typical litter and soil daily temperature profiles in forested habitat in April–June and September–October.

We randomly assigned animals to three treatment groups that were fed different numbers of 0.05-g crickets every 2 wk: low food (two crickets), medium (four crickets), and high (eight crickets). The low-food diet was intended to provide energy intake sufficient for maintenance requirements; the high-food level was not an ad libitum treatment, as all crickets were usually eaten. We measured wet mass (± 0.01 g precision) and snout–vent length (SVL, ± 0.5 mm precision) for each salamander and began feeding treatments on 17 April 1989. Container positions were randomized within the environmental chamber. Initial wet masses ranged from 3.0–6.5 g. We made no attempt to adjust food levels to the body size of individual animals. The experiment continued until 22 November 1989, when field observations showed that most females at several sites had already migrated to the breeding site, bred, and oviposited. At this time, surviving animals in the feeding experiment were weighed, dispatched, and frozen.

At a later date, animals were thawed and dissected into carcass, liver, fat body, and egg compartments. We dried body components to a constant mass at 60 C, weighed them, and placed them in pre-weighed cellulose thimbles for extraction with petroleum ether in a Soxhlet apparatus for 5 h. After extraction, thimbles were redried and weighed, and non-polar lipid amounts were determined as the difference between before- and after-extraction masses. We excluded two females in the high-food treatment from analyses as each had consistently consumed less than half of its intended food ration.

Total mass change (Δ MASS) was cal-

culated as final wet mass (including eggs in gravid females) minus initial wet mass; Δ MASS represents energy allocated during the experimental period to growth, storage, and reproduction. We calculated total lipid amount as the sum of lipid dry mass in all compartments. Lean dry mass is the difference between whole-animal dry mass and total lipids. Lipid proportions were determined as the quotient of compartmental lipid level and total lipid. Proportion data were arcsine-square root transformed prior to statistical analysis; mass variables were natural-log transformed. Untransformed data are presented in figures and text.

We used two-way, fixed-effects analysis of variance (ANOVA) models to assess the effects of food level (FOOD) and reproductive status (REPRO: gravid or non-gravid females) on Δ MASS. The ANOVA interaction term was non-significant and was incorporated into the residual error term.

We used analysis of covariance (ANCOVA) models to test for differences among food-treatment groups in lipid levels in each compartment after adjusting for an individual's initial (pre-treatment) body size. The ANCOVA used initial body mass (IMASS) as the covariate in tests for treatment effects (FOOD) on total lipid levels as well as compartmental levels in the carcass, fat bodies, liver, and eggs. In all analyses, the interaction term (FOOD*IMASS) was non-significant, indicating that the relationship between the response variable and the covariate was similar among groups; i.e., the slopes were equal. Subsequently we reduced the model to only main effects and incorporated the interaction term with the residual error.

Analysis of covariance was also used to test the effects of FOOD and final body size [final dry mass minus clutch dry mass, or the non-reproductive dry mass (NRMASS)] on clutch characters: clutch size, clutch dry mass (CLUMASS) and clutch lipids (CLULIP), and mean egg dry mass (EGGMASS) and egg lipids (EGGLIP). Analyses were conducted using the General Linear Models, Catmod, Frequency, and Regression procedures of the

TABLE 1.—Lipid levels (g dry mass, $\bar{x} \pm 1$ SE) in females of *Ambystoma opacum* from low-, medium-, and high-food treatments. Means are adjusted for initial (pre-treatment) body size by analysis of covariance. Means for the egg lipid compartment (*) were calculated using only the gravid females ($n = 1$, $n = 5$, and $n = 6$ in the low-, medium-, and high-food groups, respectively).

Compartment	Treatment		
	Low	Medium	High
Carcass	0.06 \pm 0.01	0.09 \pm 0.01	0.14 \pm 0.01
Fat bodies	0.01 \pm 0.01	0.04 \pm 0.01	0.11 \pm 0.01
Liver	0.01 \pm 0.01	0.03 \pm 0.01	0.08 \pm 0.01
Total storage	0.08 \pm 0.01	0.16 \pm 0.01	0.33 \pm 0.01
Eggs*	0.010	0.011 \pm 0.004	0.022 \pm 0.004
Total lipids	0.08 \pm 0.01	0.16 \pm 0.01	0.35 \pm 0.01

Statistical Analysis System (SAS, 1985); Type III SS were used for hypothesis tests.

RESULTS

Initial Body Sizes

The initial assignment of individuals to treatment groups successfully randomized body size among groups. Treatment groups did not differ in initial SVL ($F_{2,43} = 1.74$, $P > 0.15$) or body wet mass ($F_{2,43} = 0.11$, $P > 0.85$).

Survival during Experiment

A total of 14 females died during the experiment; the highest mortality (50%) occurred in the low-food group, with 14% in the medium group and 25% in the high-food group ($\chi^2 = 4.67$, $P = 0.10$, $df = 2$). At least five deaths appeared to be unrelated to the experimental food levels, as deaths occurred within a few weeks of the start of the experiment.

Effects on Final Body Size and Growth

We used total dry mass (TDM) at the end of the experiment as our measure of body size. Food levels significantly affected final TDM ($F_{2,26} = 162.3$, $P < 0.0001$); mean TDM ($\bar{X} \pm 1$ SE) for low, medium, and high groups after adjusting for initial body mass by ANCOVA was 0.91 ± 0.04 g, 1.20 ± 0.03 g, and 1.86 ± 0.03 g, respectively. Lean dry mass (LDM) reflects an individual's investment in growth: i.e., non-lipid investment. Food levels exerted strong effects on LDM ($F_{2,26} = 145.6$, $P < 0.0001$); adjusted means were 0.83 ± 0.03

g, 1.04 ± 0.03 g, and 1.52 ± 0.03 g for low-, medium-, and high-food treatments.

Effects on Lipid Levels

Non-polar lipids represent energy allocated to storage (and to reproduction in gravid females). Total non-polar lipid levels were affected by feeding treatments (Table 1; $F_{2,26} = 70.2$, $P < 0.0001$); the animals in the high-food treatment attained the highest lipid levels (Fig. 1). The same pattern was observed in all compartments for non-reproductive (i.e., storage) lipids. Lipid amounts in the carcass, fat bodies (Fig. 2), and liver all were highest in the high-food group animals (Table 1; $P < 0.01$ for all tests).

Proportional Allocation to Compartments

For each individual, we determined the proportion of its dry mass comprised of

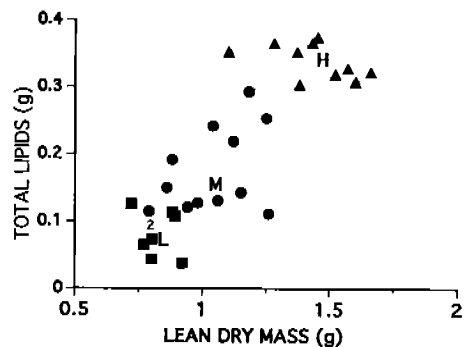


FIG. 1.—Relationship between body size (lean dry mass) and total lipid levels in females of *Ambystoma opacum* from low- (squares), medium- (circles), and high-food (triangles) level treatments. Letters represent the means for each treatment group.

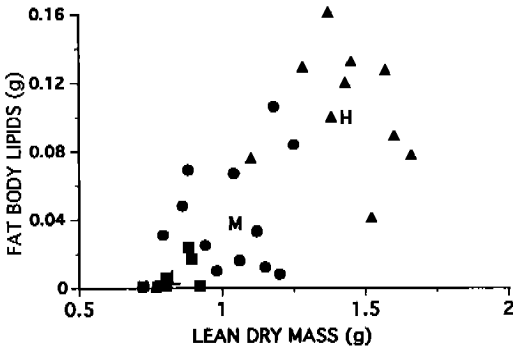


FIG. 2.—Relationship between body size (lean dry mass) and lipid levels in the fat bodies of females of *Ambystoma opacum* from low-, medium-, and high-food treatment groups. Symbols and means as in Fig. 1.

lean mass and lipids, and the proportion of its total lipids comprised of carcass, fat body, liver, and egg lipids. Food levels significantly affected the proportional allotment of lean mass and lipids ($F_{2,27} = 32.3$, $P < 0.0001$). In the low-food group, 92% of total dry mass was lean mass and 8% was lipid, compared to 85.5% and 13.5% in the medium group, and 81.5% and 18.5% in the high-food group.

Treatment groups also differed in their percentage of total lipids in each compartment. All groups exhibited the highest percentage of lipids in the carcass, and each group had approximately 20% of its lipids in the liver (Fig. 3). Groups differed in the percentage of lipids apportioned to fat bodies ($F_{2,27} = 16.6$, $P < 0.0001$) and in the carcass ($F_{2,27} = 15.5$, $P < 0.0001$; Fig. 3). Low-food females had 73% of their total lipids in carcass lipids and 6% in fat bodies; high-food females apportioned 41% of their total in carcass lipids and 31% in fat bodies.

Reproduction

Proportion reproducing.—For each treatment group, we calculated the proportion of surviving females that was gravid: i.e., showed evidence of yolked follicles at the termination of the experiment. Averaged across food-level groups, gravid females gained 30% more mass during the experiment than non-gravid females (effect of RSTATUS on Δ MASS; $F_{1,26} = 3.35$, $P < 0.08$). The proportion of females that

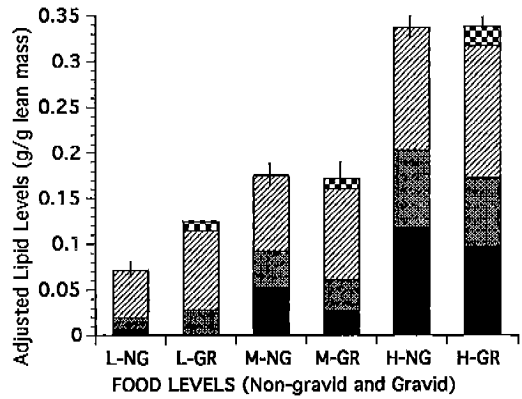


FIG. 3.—Proportional allotment of lipids in carcass, liver, fat body, and reproductive compartments in non-gravid (NG) and gravid (GR) females of *Ambystoma opacum* from three experimental feeding regimes: low- (L), medium- (M), and high-food (H) levels. Bar sections (from bottom to top) represent fatbody, liver, carcass, and clutch lipid levels, respectively. Vertical lines represent 1 SE of the total lipid level means.

became gravid increased significantly as food levels increased (Fig. 4: Mantel-Haenszel ordered chi-square, $\chi^2 = 3.98$, $P < 0.05$, $df = 1$).

Clutch size relationships.—Only one female in the low-food group was gravid, so we limited subsequent analyses of clutch size and egg size relationships to medium- and high-food groups. In analyses that involved body size, we used non-reproductive dry mass (NRDM) as our measure of size, because it reflects a female's total allocation to growth and storage.

We observed a positive relationship between clutch size and NRDM ($r = 0.71$, $P < 0.02$, $n = 11$; Fig. 5). Food level significantly affected clutch size (CS; $F_{1,9} = 10.1$, $P \leq 0.01$); high-food females had larger clutches (Fig. 5). However, after removing the effect of NRDM, there was no effect of food level on CS. Clutch mass (CLUMASS) was weakly correlated with NRDM ($r = 0.53$, $P \leq 0.09$, $n = 11$). However, treatment groups did not differ in relative clutch mass (RCM); there was no effect of food level on clutch mass after adjusting for NRDM ($F_{1,8} = 0.30$, $P > 0.60$). A positive relationship existed between clutch lipids (CLULIP) and NRDM ($r = 0.63$, P

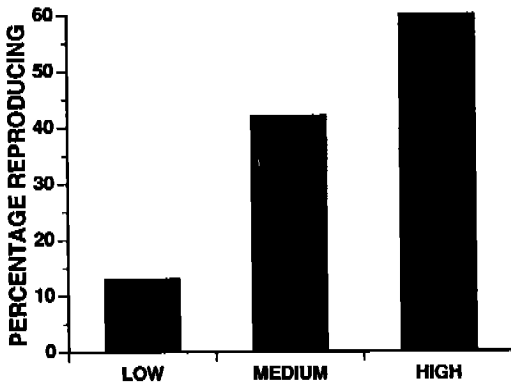


FIG. 4.—The percentage of surviving females reared for 232 days at three food levels that exhibited yolked follicles at the end of the experiment. Total number was eight females in the low-food group, 12 in the medium food group, and 10 in the high food group.

< 0.04, $df = 11$), but we observed no effect ($F_{1,8} = 0.16$, $P > 0.70$) of FOOD on CLULIP apart from the food-level effect on body size.

Egg lipids and egg size.—There was no correlation between mean lipid investment per egg (EGGLIP) or mean individual egg dry mass (EGGDM) and NRDM, nor was there any effect of food level on EGGLIP ($F_{1,8} = 1.43$, $P > 0.25$) or EGGDM ($F_{1,8} = 1.80$, $P > 0.20$).

Residuals analysis.—We regressed clutch size, clutch lipids, and egg lipids on NRDM, and examined relationships among residuals. Clutch size residuals (Fig. 6) were positively correlated with CLULIP residuals, indicating that females with relatively high reproductive-lipid levels for their body size tended to have larger clutches ($r = 0.80$, $P < 0.01$, $n = 11$). Egg lipid residuals were not correlated with CLULIP residuals ($r = 0.42$, $P > 0.20$, $n = 11$); females with relatively large clutch-lipid reserves for their body size did not appear to make relatively larger eggs. Egg lipid and CS residuals were not correlated ($r = 0.38$, $P > 0.25$, $n = 11$); females with larger clutches did not invest more lipid per egg (Fig. 7). Interpretation of these results may be equivocal if egg formation was not completed at the time that females were killed (see Discussion).

We conducted similar analyses for 25

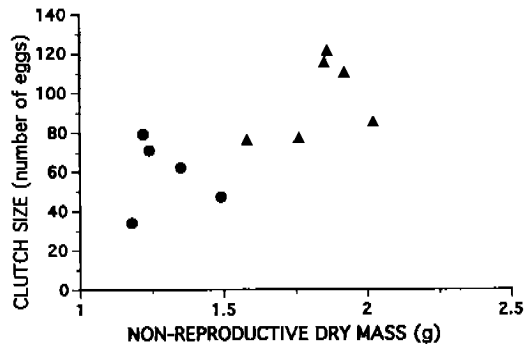


FIG. 5.—The relationship between body size (non-reproductive dry mass) and clutch size in females of *Ambystoma opacum* from the medium- and high-food level groups. Circles represent medium-food, triangles the high-food females.

gravid females collected from Ginger's Bay in a separate study (Scott, 1994; D. E. Scott, unpublished data). Clutch size residuals in these wild-caught females also were positively correlated with CLULIP residuals ($r = 0.56$, $P < 0.01$), but EGGLIP residuals were not ($r = 0.33$, $P > 0.10$), as was observed in the feeding-experiment females. In the wild-caught females, however, we observed a significant, negative correlation between CS and EGGLIP residuals ($r = -0.55$, $P < 0.01$); for a given body size, females that had relatively large clutches had relatively small eggs.

Clutch relationships after removal of

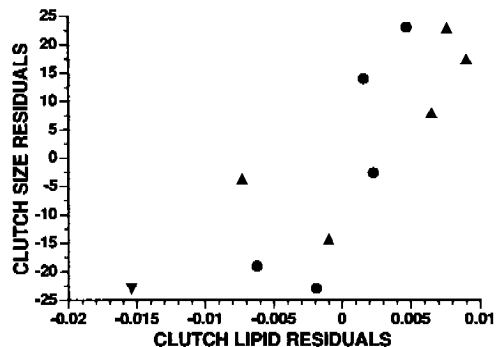


FIG. 6.—Relationship between clutch size residuals and clutch lipid residuals for females from two experimental food levels. Residuals for both variables were determined from a regression of clutch size and clutch lipids on female non-reproductive dry mass. Symbols as in Fig. 5; inverted triangle represents a high-food female that is a potential outlier.

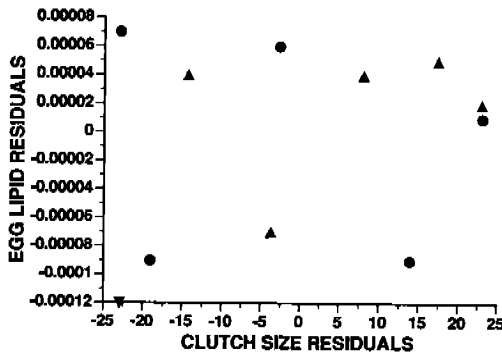


FIG. 7.—Relationship between egg lipid residuals and clutch size residuals determined from a regression of both variables on female non-reproductive dry mass. Symbols as in Fig. 6.

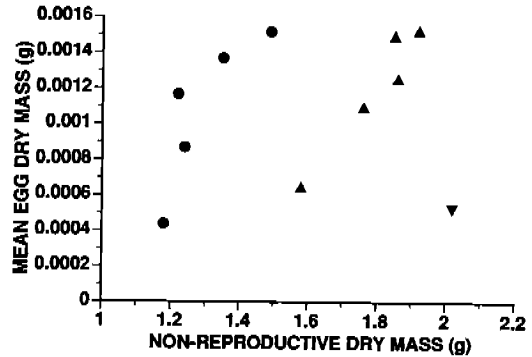


FIG. 8.—Relationship between mean egg size (dry mass) and body size in females of *Ambystoma opacum* from medium- and high-food treatments. Symbols are as in Fig. 6.

outlying data point.—The clutch and egg analyses summarized above were highly dependent on one data point, a 2.0-g dry mass female with extremely small eggs and clutch mass for her body size (Fig. 8). Exclusion of this animal changed some, but not all, of the relationships observed between NRDM, FOOD, and clutch characteristics. Correlations of CLULIP and CLUMASS with NRDM were stronger ($r = 0.77$ and 0.72 , respectively; $P < 0.02$, $n = 10$). Food-level means, adjusted for body size, did not differ for either CLULIP ($F_{1,7} = 1.10$, $P > 0.30$) or CLUMASS ($F_{1,7} = 1.65$, $P > 0.20$).

In the ANCOVA, both egg lipids and egg size were affected by NRDM and FOOD. Egg lipid was strongly correlated with NRDM ($r = 0.67$, $P < 0.05$, $n = 10$). After adjusting for body size by ANCOVA, FOOD also affected EGGLIP ($F_{1,7} = 8.63$, $P \leq 0.02$); adjusted egg lipid means were over two times larger in the medium-food group. Similarly, EGGDM was related to NRDM ($F_{1,7} = 18.34$, $P < 0.01$; Fig. 8) and differed between food groups ($F_{1,7} = 11.85$, $P \leq 0.01$). Adjusted egg size in medium-food females was over three times larger than in high-food females. Mean egg lipid and egg dry mass, unadjusted for female body size, did not differ between food levels ($F_{1,8} \leq 1.26$, $P > 0.25$).

DISCUSSION

In this study, we observed food-level effects on laboratory survival, growth, lipid

allocation, and reproductive traits in *Ambystoma opacum*. The relationship between dietary level (i.e., caloric intake) and survival is straightforward; obviously, starvation of animals in the low-food group was not our intent. Prior to the experiment, we estimated metabolic rates of average-sized females (3.5–4.5 g) held at 15 C, and we calculated an estimate of caloric expenditure of ~70–105 cal per week. The caloric content of the size crickets that we fed to the salamanders was ~50–100 cal/cricket (estimated from Vogel et al., 1986). However, some low-food animals apparently died because caloric intake was insufficient to maintain the animal.

The primary response observed in this study was increased growth of females at higher food levels. All other responses appeared to be a function of the increases in body size; i.e., including final female body size as a covariate tended to mask the effects of food level. For example, wild-caught females exhibit increases in the percentage of their dry mass comprised by lipids as body size increases (D. E. Scott, unpublished data). Allocation patterns in females from the feeding experiment (Figs. 1–3) were similar to wild-caught females. Increased food intake resulted in larger body size and changes in the proportional allocation to lipid compartments as a function of body size.

Post-breeding females of *A. opacum* leave the breeding site with <2% of their dry mass in lipid stores and >98% as lean

mass (D. E. Scott, unpublished data). In the low-food group, surviving females increased their proportion of fat reserves only slightly from post-breeding to the next breeding period; more of the food in excess of maintenance demands was allocated to increase lean dry mass (growth) as opposed to lipid levels (storage). In medium- and high-food groups, proportionally more of the excess was partitioned into storage and reproductive lipids. In particular, females in these higher food-level groups coupled lean mass growth with increases in fat body lipids; i.e., there was simultaneous allocation to growth, reproduction, and storage. Larger salamanders probably have decreased size-specific metabolic demands, and as the proportion of overall energy intake devoted to maintenance costs goes down in larger animals, the proportion available for storage and reproduction may increase. In order for a female to reproduce, it is likely that her fat body lipids must be replenished; numerous studies have implicated the importance of fat bodies in vitellogenesis (e.g., Hahn and Tinkle, 1965; Rose and Lewis, 1968). Female toads (*Bufo bufo*) with depleted fat reserves did not exhibit growth in oocytes until individuals were fed and attained a good nutritional state (Jørgensen, 1967).

In organisms with indeterminate growth, the relationship between growth and reproduction is important because size is positively correlated with fecundity. The larger clutch sizes observed in high-food females were due primarily to increases in body size; after adjusting for body size, differences between groups disappeared. Food level contributes to size-specific variation in clutch size through its effect on a female's allocation to yolk materials. Food level strongly affected clutch lipids and clutch dry mass, and analysis of clutch size and clutch lipid residuals revealed that a female with a relatively large reservoir of yolk material for her body size will make more eggs.

Clutch size variation among same-sized females may also be related to egg size differences. In amphibians, individuals with large size-specific clutch size may also have relatively small size-specific egg size (Jørgensen, 1981; Kaplan and Salthe, 1979).

In the natural population of *A. opacum* at Ginger's Bay, females that had relatively large clutches for their body size also had relatively small eggs. However, our experimental results pertaining to the effects of food level on egg size are equivocal for several reasons. First, sizes of the yolked follicles in this study (0.2–2 mg dry mass) were smaller than yolked follicles in wild-caught females (3–3.5 mg), and females in natural populations also exhibit less variation in egg size (Kaplan, 1980a,b). Thus, dietary deficiencies may have caused abnormally small eggs (Jørgensen, 1992), although *Rana pipiens* fed crickets ad lib exhibited extensive gain in mass and developed normal-sized eggs (Lehman, 1978). Additionally, the artificial-light regimen may have altered vitellogenic growth (Jørgensen, 1992). After 8 mo of captivity, the ovarian cycle may not have been synchronous with the natural populations nearby, and vitellogenesis may not have been complete at the termination of the study.

Second, even if absolute amounts of yolk deposition are presumed to be low but the relative relationships between groups is unaffected, interpretation of the effect of food level on egg size is influenced heavily by one data point. Other studies have demonstrated a positive relationship between egg size and body size in *A. opacum* (Kaplan, 1980a,b; D. E. Scott, unpublished data). If all of the 11 gravid females from the medium- and high-food treatments are included, then egg size and egg lipids were unrelated to food level and body size. Only when the potential outlier is removed did we observe a correlation between body size and egg size in this study, and an effect of food level on egg lipids. Relative egg size (adjusted for female body size) is strongly affected by food level. Larger females produce larger eggs, but females with lower food intake produce relatively larger eggs than females given higher food levels, after adjusting for differences in body size. Reznick and Yang (1993) reported similar observations for offspring from female guppies (*Poecilia reticulata*). Females given lower food amounts had lower fat reserves, but they allotted relatively more fat to each embryo (Reznick and Yang, 1993).

Egg size in amphibians is strongly linked to offspring fitness. Variation in egg size is related to hatchling size and often to subsequent hatchling performance. Several studies have documented positive correlations between egg volume, hatchling size, larval size at first feeding, time to first feeding, and size at metamorphosis (Kaplan, 1985, 1987, 1989). Food-level effects on absolute (unadjusted) egg size or egg lipids mediated through effects on body size may potentially influence an individual's larval success.

Several studies have documented minimal effects of food level on egg size (Berven, 1982; Ford and Seigel, 1989). However, others have reported significant plasticity in egg size (Ballinger, 1977; Kaplan, 1987). In *A. opacum*, observed among-female variation in egg size (Kaplan, 1980a,b) probably stems largely from variation in body size and also genetic differences in metabolic rate or any of the numerous mechanisms that influence yolk deposition in follicles: rates of vitellogenesis, cellular transport mechanisms, and ovarian capillary networks, for example (Follett and Redshaw, 1974). Despite food-level effects on relative egg size (after adjusting for body size), it appears that absolute egg size exhibits little plasticity in *A. opacum*; i.e., there is evidence for canalization in egg size. Egg size is strongly coupled to offspring size, and it is a reproductive trait on which selection presumably would act strongly. Experiments that subject individual females to different food levels (e.g., Kaplan, 1987) represent one of the best means to determine the degree of plasticity in egg size, but such experiments pose difficulties in salamanders.

Phenotypic plasticity resulting from environmental factors, such as prey availability, is an important component of life-history variation in reproductive traits (Ballinger, 1977; Ford and Seigel, 1989; Kaplan, 1987). Meaningful interpretation of the evolution of life-history patterns is predicated on knowing the distinction between environmentally induced variation and genotypic variation (Berven, 1982; Stearns, 1977). In amphibians, age and size at maturity are two traits that exhibit plas-

ticity and are modulated, at least in part, by environmental conditions (Bernardo, 1993; Berven, 1982; Scott, 1994). Our experiment demonstrates that other traits (i.e., growth, lipid storage, and reproductive traits) in *Ambystoma opacum* also are influenced by proximate environmental factors. Local food abundance affects the life-history phenotype on which selection acts, and thereby influences an individual's fitness apart from any genetic differences among individuals in life-history traits.

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