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Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival

David E. Scott · Erin D. Casey · Michele F. Donovan · Tracy K. Lynch

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Abstract In organisms that have complex life cycles, factors in the larval environment may affect both larval and adult traits. For amphibians, the postmetamorphic transition from the aquatic environment to terrestrial habitat may be a period of high juvenile mortality. We hypothesized that lipid stores at metamorphosis may affect an animal's success during this critical transition period. We examined variation in total lipid levels among years and sites in recently metamorphosed individuals of two pond-breeding salamander species, the marbled salamander (*Ambystoma opacum*) and the mole salamander (*A. talpoideum*), with limited data for one anuran species (southern leopard frog, *Rana sphenocephala*). Lipid levels were allometrically related to body size and ranged from 1.9 to 23.8% of body dry mass. The two salamander species differed in lipid allocation patterns, with *A. opacum* apportioning a higher percentage of total lipid reserves into fat bodies than *A. talpoideum*. Species differences in lipid allocation patterns may primarily reflect that large metamorphs will mature as one-year olds, and, regardless of species, will alter lipid compartmentalization accordingly. We used mark–recapture data obtained at drift fences encircling breeding ponds for 13 *A. opacum* cohorts to estimate the proportion of postmetamorphic individuals that survived to breed (age 1–4) and the mean age at first reproduction. Regression models indicated that size-corrected lipid level at metamorphosis (i.e., lipid residuals), and to a lesser extent rainfall following metamorphosis, was positively related to adult survival. Snout-vent length at metamorphosis was negatively related to age at first reproduction. We suggest that lipid stores at metamorphosis are vital to juvenile survival in the months following the transition from aquatic to terrestrial habitat, and that a trade-off shaped by postmetamorphic selection in the terrestrial habitat exists between allocation to energy stores versus structural growth in the larval environment.

Keywords Carryover effects · Fitness correlates · Lipids · Postmetamorphic survival · Trade-offs

Introduction

In organisms with complex life cycles (e.g., many marine invertebrate, insect, fish, and amphibian species), factors in the larval environment have strong carryover effects on juveniles and adults (Pechenik 2006). For example, food limitation and crowding in the larval phase affect postmetamorphic traits in *Drosophila* (Prout and McChesney 1985), marine gastropods (Pechenik et al. 1998), barnacles (Jarrett 2003), mussels (Philips 2002, 2004), stoneflies (Peckarsky and Cowan 1991; Taylor et al. 1998), damselflies (Anholt 1991), marine (Booth and Hixon 1999) and freshwater fish (Ward and Slaney 1988), and amphibians (e.g., Scott 1994; Altwegg 2003). The common environment experienced by an entire cohort of larvae may lead to delayed life-history effects with consequences for population dynamics of adults (Beckerman et al. 2002), as adult traits (e.g., survival, growth, size and age at maturity) are influenced by

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D. E. Scott (✉) · E. D. Casey · M. F. Donovan ·
T. K. Lynch
Savannah River Ecology Laboratory,
P.O. Drawer E, Aiken, SC 29802, USA
e-mail: scott@srel.edu

factors long before adulthood is reached. Metamorph quality, in conjunction with metamorph numbers and postmetamorphic environmental conditions, combine to shape a species' population dynamics.

An understanding of metamorph quality requires knowledge of how assimilated food energy is allocated. Resources allocated to structural growth are unavailable for other, competing, processes of maintenance and reproduction; i.e., allocation trade-offs occur (Perrin and Sibley 1993). Energy allocation decisions are the cornerstone of life-history theory (Stearns 1992), and these trade-offs presumably occur in most animals due to finite food availability (Congdon 1989). Resource allocation decisions collectively affect an organism's survival probability and reproduction, and thus its fitness (Stearns 1992). Natural selection should favor individuals that appropriately allocate their assimilated energy among the competing compartments of maintenance, structural growth, reproduction, and storage for future allocation (Bernardo 1994).

The storage compartment of an organism's energy budget is its lipid reserve, which in amphibians is usually located in discrete abdominal fat bodies or reserves in the tail (Fitzpatrick 1976; Pond 1978). In ectotherms, lipids are used for gonadal development and as energy reserves during hibernation or estivation. Given the environmental constraints on periods when many amphibians can be actively feeding, lipid reserves serve to stave off starvation (Pinder et al. 1992). For example, in the mountain yellow-legged frog (*Rana muscosa*), between-year recapture rates were higher for frogs of high relative mass, presumably reflecting the overwinter survival advantage of fatter individuals (Pope and Matthews 2002).

In general, most studies of lipid utilization in amphibians have examined adult patterns (e.g., Fitzpatrick 1976; Seymour 1973), although some have examined lipid use during the metamorphic climax (Beck and Congdon 2003, and references therein). Variation in larval food resources, densities, and other factors in aquatic environments promote differences in lipid reserves at metamorphosis. Crump (1981) documented that larval density affects energy reserves at metamorphosis in *Hyla crucifer*, and proposed that individual fitness is related to body size through effects on fat stores. Pfennig (1992) noted that pond permanency affects reserves in spadefoot toads (*Scaphiopus multiplicatus*), and high larval density caused small size and low fat reserves at metamorphosis in the marbled salamander, *Ambystoma opacum* (Scott 1994). Short-term survival under laboratory conditions was associated with juvenile body size and energy reserves in both *S. multiplicatus* and *A. opacum* (Pfennig 1992; Pfennig et al. 1991; Scott 1994). If lipid stores enhance postmetamorphic survival in field populations, then it would be expected that energy allocation would shift in larval amphibians from growth to storage once adequate body size has been attained, to reduce risk of predation and/or enable metamorphosis. Variation in allocation patterns resulting from differences in the larval growth environment should be reflected by among-cohort variation in lipid levels at metamorphosis.

We examined lipid levels at metamorphosis in three species of amphibians from four ponds over seven years. Our objectives were to: (1) document within- and among-species variations in total lipid levels in relation to body size, (2) examine variation between two salamander species in energy partitioning to fat bodies versus other storage compartments, and (3) use mark–recapture data available for salamanders to describe the relationships among larval environment (larval density and pond duration), metamorph body size and lipid levels, postmetamorphic terrestrial habitat severity (using rainfall as a proxy for environmental severity), and mean age at and survival to first reproduction. These results allowed us to assess potential trade-offs between allocation to energy stores versus structural growth in the larval environment, and the carryover effects these allocation decisions have on adult traits related to fitness.

Materials and methods

Field collections

We used drift fences and pitfall traps (Scott 1994) to capture newly metamorphosed individuals of the marbled salamander (*Ambystoma opacum*), mole salamander (*A. talpoideum*) and southern leopard frog (*Rana sphenocephala*) as they exited their natal ponds. Five to 62 individuals of each population were collected in the field, euthanized in the laboratory, and frozen at –60 °C. Collections were irregular depending upon the species, site, and year. Additional collections of *A. opacum* metamorphs were made from experimentally manipulated ''pen populations'' at three sites; summary data for *A. opacum* from two sites and six density treatments were reported previously (Scott 1990, 1994). The total number of populations presented here are: 25 for *A. opacum* (15 pen populations, ten whole wetland populations), six *A. talpoideum*, and three *R. sphenocephala*; the data used in each analysis are presented in Table 1. Breeding ponds were located in Aiken and Barnwell counties on the US Department of Energy's Savannah River Site in South Carolina. Aquatic habitats are described elsewhere for three sites: Rainbow Bay (RB; Semlitsch et al. 1996), Ginger's Bay (GB) and Bullfrog Pond (BP; Scott 1990). The fourth site, Squirrel Bay (SB), is an herbaceous wetland similar in size (<1 ha) to GB and RB that is dominated by an aquatic grass, *Panicum hemitomon*. In general, SB holds water longer than GB, BP, and RB.

Table 1 Summary of species cohorts used for each analysis

Species	Site	Year	Cohort type	Used in analysis of
A. opacum	GB	1986	LD (2)	LDM; lipid; full; rain; larval
	GB	1986	HD (3)	LDM; lipid; full; rain; larval
	RB	1986	Natural	Lipid; comp; full; rain; larval
	GB	1987	LD (3)	LDM; lipid; full; rain; larval
	GB	1987	HD (3)	LDM; lipid; full; rain; larval
	BFP	1987	LD (2)	LDM; lipid; larval
	BFP	1987	HD (2)	LDM; lipid; larval
	SB	1989	Natural	Lipid; comp
	GB	1989	Natural	Lipid; comp; full; rain; larval
	GB	1990	Natural	Lipid; comp; full; rain; larval
	RB	1990	Natural	Rain
	GB	1991	Natural	Lipid; comp; full; rain; larval
	RB	1991	Natural	Lipid; comp; full; rain; larval
	GB	1993	Natural	Lipid; comp; full; rain; larval
	RB	1993	Natural	Lipid; comp; full; rain; larval
	GB	1994	Natural	Lipid; comp; full; rain; larval
	RB	1994	Natural	Lipid; comp; full; rain; larval
	RB	8 years	Natural	Hydroperiod effect on SVL only
A. talpoideum	RB	1987	Natural	Lipid; reduced
	RB	1991	Natural	Lipid; comp; reduced
	GB	1991	Natural	Lipid; comp
	RB	1993	Natural	Lipid; comp; reduced
	GB	1993	Natural	Lipid; comp
	GB	1994	Natural	Lipid; comp
R. sphenocephala	GB	1991	Natural	Lipid
	RB	1991	Natural	Lipid
	EB	1991	Natural	Lipid

Analyses are indicated as *LDM* (lean dry mass to snout-vent length regression), *lipid* (total lipids to lean dry mass regression), comp (analysis of covariance to compare allocation between salamanders), *full* (full multiple regression model—effects of size-adjusted lipid levels, SVL, and postmetamorphic rainfall on survival and mean age at first reproduction), *reduced* (reduced regression model—effect of SVL on survival and mean age at first reproduction), *rain* (rainfall to postmetamorphic survival regression), and *larval* (multiple regression—effects of wetland hydroperiod and larval density on metamorph traits). Number of pen populations for low-density (LD) and high-density (HD) experimental treatments (Scott 1990) shown in parentheses; *pen* means used for LDM and lipid analyses, *treatment* means in full, rain, and larval analyses

Lipid extractions

Samples were thawed and dissected prior to lipid analysis. We performed nonpolar lipid extractions using a Soxhlet apparatus with petroleum ether as a solvent (Dobush et al. 1985). Samples were cut into small pieces, oven-dried at 60 °C for 5–7 days to a constant mass, ground with mortar and pestle, placed in preweighed cellulose thimbles, and weighed before and after extraction to ±0.1 mg. Samples were extracted for 5–6 h, and lipid amounts were calculated as the dry mass lost during extraction. For the whole-wetland populations of salamanders, we removed fat bodies from a subset of animals (6–17 animals for five *A. talpoideum* populations, 6–53 for ten *A. opacum* populations) and separately extracted the fat body and remaining carcass samples, which allowed us to compare lipid allocation to fat bodies versus carcass.

The lean dry mass (LDM) of each individual was determined as the difference between whole animal dry mass and total lipids (TL); percent lipids was calculated as (TL/total dry mass) × 100. We used LDM as a measure of body size in some analyses when snout-vent length (SVL) measures were not available; LDM is highly correlated with SVL (see ‘‘Results’’) and it also represents an individual’s investment in structural growth rather than energy storage.

Mark–recapture techniques

Ginger’s Bay and RB are encircled by drift fences, which allowed us to examine cohorts through time as animals matured and returned to natal ponds as breeding adults. In general, captured amphibians were sexed, measured (SVL, ±0.5 mm), toe-clipped for identification, and released on the opposite site of the drift fence. Most data reported in this study are from juveniles that were cohort-marked (Ott and Scott 1999) with a year-specific mark as they emigrated from their natal wetlands. Recapture data were used to assess relationships among metamorph traits (SVL and TL), cumulative rainfall immediately following metamorphosis, and adult survival to and mean age at first reproduction in 13 *A. opacum* and three *A. talpoideum* cohorts. For breeding adult ambystomatid salamanders at GB and RB the drift fence trespass rates are <5% (DES unpublished data); therefore, the catchability is high and if previously marked animals return to the wetland they are very likely to be captured.

Estimating survival to age 4

Males and females generally reach first reproduction at different ages (Pechmann 1994), and survival to first reproduction also varies among cohorts (Scott 1994), but the majority of surviving, nondispersing individuals of both sexes return to breed by age 4 (Semlitsch et al. 1988; Scott 1994, 2005). For each salamander cohort the total number of first-time breeders that returned by age 4 (N_{BF}) was used to calculate our estimate of survival ($S_{1\text{–}4}$). At GB, determining N_{BF} was straightforward, as we clipped an additional single toe on cohort-marked animals at each breeding season, which allowed us to distinguish first time breeders at each breeding season through age 4. At RB, additional toes were not clipped each season on cohort-marked animals, so we could not distinguish first-time breeders from repeat breeders for two-, three-, and four-year-olds. However, at RB additional data from individually marked juveniles were available to estimate N_{BF} in cohort-marked animals (Semlitsch et al. 1996). For RB cohort-marked animals we: (1) summed the total number of breeding adult captures (N_{BT}) in years 1–4, which included some individuals that may have bred two or more times, (2) used data from individually marked RB animals to determine the proportion of total captures represented by first-time breeders ($N_{\mathrm{BF}}/N_{\mathrm{BT}}$), and (3) multiplied the total captures of cohort-marked animals by this proportion to estimate N_{BF} for the cohort-marked animals. We estimated survival through age 4 for all cohorts as:

$$S_{1-4} = N_{\mathrm{BF}}/N_{\mathrm{M}}, \tag{1}$$

where N_{M} represents the total number of marked metamorphosed juveniles for that cohort. For example, in 1986 at RB 1,190 *A. opacum* metamorphs were individually marked, of which 73 individuals were recaptured 81 times at ages 1–4 (i.e., 89% were first-time breeders). In the same year at RB 1,100 metamorphs were cohort-marked, and 13 total breeding adult recaptures were recorded over the following four years; N_{BF} for this cohort-marked group was estimated as $13 \times 0.89 = 12$, and $S_{1\text{–}4} = 12/1{,}100 = 0.01$. Complete data from nine *A. opacum* cohorts from GB (summary data on four of these were presented in Scott 1994) and four from RB were used in survival analyses. For *A. talpoideum*, complete mark–recapture data were only available for three RB cohorts, which precluded most statistical analyses.

Estimating mean age at first reproduction

Mean age at first reproduction was determined for 13 *A. opacum* and three *A. talpoideum* cohorts. As in the analyses of survival, RB cohort data were supplemented with data from individually marked animals to allow us to determine the fraction of the total breeding population each year represented by first-time breeders. Cohort mean age at first reproduction (A) was calculated as:

$$A = [N_{\mathrm{BF1}} + 2(N_{\mathrm{BF2}}) + 3(N_{\mathrm{BF3}}) + 4(N_{\mathrm{BF4}})]/N_{\mathrm{BF}}, \tag{2}$$

where $N_{\mathrm{BF}i}$ is the number of first time breeders at each age (1–4 years).

Environmental variables

Larval density and pond hydroperiod were used as independent variables in an analysis of lipid and SVL variation in newly metamorphosed *A. opacum*. Density was determined directly (in experimentally manipulated populations) or calculated for whole-wetland populations by dividing the estimated number of hatchlings (assuming a clutch size of 100 eggs/female and a hatch success of 50%) by the maximum filled area of the wetland in each year. Hydroperiod was measured as the number of consecutive days the wetland held water during the primary winter/spring period of filling. Total daily rainfall was measured (±0.5 mm) by a gauge at RB. For the analysis of post-metamorphic survival of juveniles, we determined the median date of metamorphosis for each cohort, which in these field populations is generally skewed toward the beginning of the metamorphosis ‘‘window’’ (i.e., 50% of the metamorphs in a cohort are generally captured in the first few night pulses of emigrants). We calculated cumulative rainfall for a three-week period following the median date of metamorphosis as an indicator of how favorable the environmental/feeding conditions for juveniles were immediately following metamorphosis, as laboratory survival depending solely on fat stores for *A. opacum* is 3–5 weeks (Scott 1994).

Statistical analyses

We used year-specific population (= cohort) means for all analyses, based on the premise that individuals within a pond in a given year were not independent observations; for data from experimental density manipulations (i.e., GB data in 1986 and 1987, BP in 1987), we treated each enclosure as a ‘‘population’’ to analyze the relationship between LDM and SVL, as well as TL and LDM. Because larval environment at a site differs dramatically from one year to the next (i.e., different physical variables, numbers of breeding females, food resources, hydroperiod, etc.), we treated populations of metamorphs in different years as independent observations, and did not account for time in the analyses. Proportion data were arcsine-square-root-transformed and other variables were log-transformed prior to statistical analyses; untransformed data are presented in figures and text. We initially explored within-species relationships among variables by examining Pearson product–moment correlation coefficients. Analysis of covariance (ANCOVA) was used to examine salamander species differences in lipid allocation patterns; after first testing for a species × body size interaction (which was nonsignificant in all cases), the interaction term was deleted from the ANCOVA model. Multiple regression models were used to test effects of metamorph SVL, TL, and postmetamorphic rainfall on adult survival to (S_{1-4}) and age at (A) first reproduction. For these models we first regressed TL on SVL and output the lipid residuals; lipid residuals reflected whether cohorts were relatively fat or lean for their mean body size. Lipid residuals, SVL, and rainfall were then used in regression analyses with S_{1-4} and A as response variables. We also regressed S_{1-4} on SVL and output the survival residuals, which reflected whether cohorts survived relatively well or poorly for their mean body size. We analyzed these two size-adjusted variables to test whether cohorts that were fat for their size corresponded to those that survived well for their size. Only the data set for *A. opacum* had enough observations to use all three independent variables in the regression model; for *A. talpoideum* our analyses were restricted to a single independent parameter (see analysis summary in Table 1). Analyses were performed using SAS for Windows v9.1 (SAS Institute 2003).

Results

Relationship of total lipids to body size

Newly metamorphosed marbled salamanders from experimental density manipulations (i.e., 15 pen populations) at GB (1986 and1987) and BP (1987) were used to examine the relationship between SVL and LDM. Lean dry mass was a good predictor of SVL ($R^2 = 0.96$, $F_{(1,13)} = 286.9$, $P < 0.0001$), and in subsequent lipid analyses we used LDM as our estimate of individual body size in instances when we did not have SVL data.

The ANCOVA for species differences in total lipid levels revealed that: (1) the TL versus LDM relationship was similar for all species (species × LDM interaction, $F_{(2,28)} = 0.4$, $P > 0.7$), (2) species did not differ in total lipid levels adjusted for body mass ($F_{(2,30)} = 1.3$, $P > 0.2$), and (3) TL was strongly related to LDM ($F_{(1,30)} = 569.4$, $P < 0.0001$; Fig. 1). For *A. opacum* metamorphs ($N = 419$) combined from 15 pen and 10 natural wetland populations, LDM explained 95% of the variance in TL ($F_{(1,23)} = 447.7$, $P < 0.0001$). In *A. talpoideum*, LDM of 94 metamorphs from six wetland populations accounted for 95% of the variance in TL ($F_{(1,4)} = 78.6$, $P < 0.001$). The relationship between body size and TL in *Rana sphenocephala* was also strong ($R^2 = 0.98$), but nonsignificant due to the sample size of only three wetland cohorts ($N = 25$ individuals sampled in a single year; $F_{(1,1)} = 53.3$, $P = 0.087$).

Total lipids exhibited a positive allometry with LDM; i.e., the slope of the regression of log TL on log LDM was significantly greater than one for both *A. opacum* (slope = 2.64 ± 0.12) and *A. talpoideum* (slope = 2.24 ± 0.25), indicating that percent lipids was greater in larger animals. Lipids ranged from 1.0 to 15.1% of LDM in *A. opacum*, 2.8–23.8% in *A. talpoideum*, and 13.2–17.8% in *R. sphenocephala*.

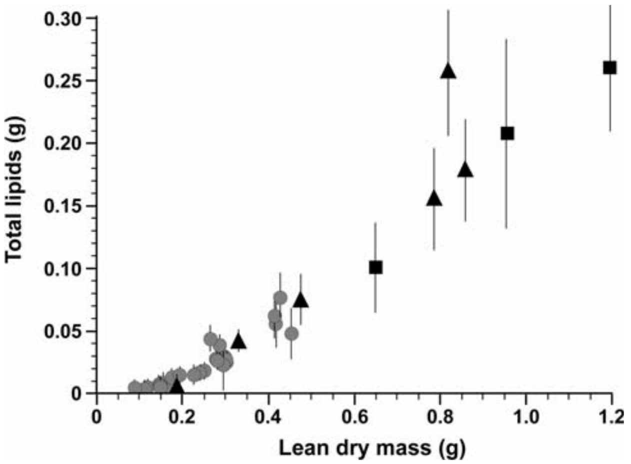

Fig. 1 Relationship between size at metamorphosis (lean dry mass) and total lipid levels in *Ambystoma opacum* (*filled gray circles*), *A. talpoideum* (*filled triangles*), and *Rana sphenocephala* (*filled squares*) from experimental and natural populations at four wetlands from 1986 to 1994. Data points represent mean values ±1 SD ($N = 5$–62 individuals) for each population

Between-species variation in lipid allocation to fat bodies versus carcass

We examined how lipid allocation patterns relate to body size in *A. opacum* and *A. talpoideum* (Fig. 2). Fat body lipid (FBL) levels increased with body size, as did carcass lipids (*A. opacum*: FBL, $F_{(1,8)} = 27.1$; $P < 0.001$, CL, $F_{(1,8)} = 77.3$; $P < 0.001$; *A. talpoideum*: FBL,$F_{(1,3)} = 45.9$; $P < 0.01$; CL, $F_{(1,3)} = 35.7$, $P < 0.01$).

ANCOVA confirmed the significant relationship between all lipid measures and LDM (i.e., CL, FBL, and TL all increased with body size; all P values < 0.0001), but revealed a difference between salamander species in lipid allocation. After accounting for body size, there was no difference between species in TL. However, size-adjusted FBL levels differed, with larger fat body lipid reserves in *A. opacum* compared to similar-sized *A. talpoideum* ($F_{(1,12)} = 5.2$, $P < 0.05$). Adjusting for body size differences, *A. opacum* apportioned 35.6 ± 2.1% of total lipids to fat bodies, and *A. talpoideum* only 17.1 ± 3.3%.

Environmental factors influencing metamorph and adult traits

Correlations among environmental variables (density, hydroperiod, postmetamorphic rainfall), metamorph traits (TL and SVL), and adult fitness measures ($S_{1\text{–}4}$ and A) for *A. opacum* are presented in Table 2. In the aquatic environment, larval density was negatively correlated with metamorph size and lipids, and positively related to adult age at first reproduction. Larger SVL at metamorphosis was associated with longer hydroperiods, and high adult survival occurred with high postmetamorphic rainfall. Adult survival also increased as lipid levels at metamorphosis rose (Fig. 3). Multiple regression models showed that larval density and hydroperiod explained 68% of the variance in lipid stores of metamorphs ($F_{(2,12)} = 12.9$, $P < 0.001$), due to a highly significant negative relationship between density and TL ($t = –5.07$, $P < 0.001$). The density/hydroperiod model was a good predictor of SVL at metamorphosis ($F_{(2,20)} = 17.5$, $P < 0.001$, $R^2 = 0.64$), with a positive relationship of hydroperiod ($t = 3.1$, $P < 0.01$) and a negative relationship of larval density ($t = –4.45$, $P < 0.001$) to SVL.

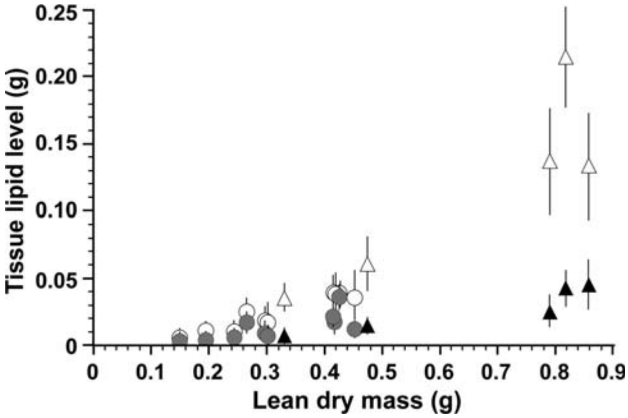

Fig. 2 Relationship between body size at metamorphosis (lean dry mass) and tissue lipid levels in *Ambystoma opacum* (*open circle*, *filled gray circles*) and *A. talpoideum* (*open triangles*, *filled triangles*) from three wetlands from 1986 to 1994. *Open symbols* depict lipid levels from carcass; *closed symbols* represent fat body lipid stores. Data points represent mean values ±1 SD ($N = 5–53$ individuals) for cohorts from each wetland and year

Relationship of metamorph body size, lipid residuals, and postmetamorphic rainfall to measures of adult fitness

The median date of metamorphosis for *A. opacum* varied from April 20 (1991 at RB) to May 22 (1987 at GB), with cumulative rainfall in the subsequent three weeks ranging from 8.8 mm (1986) to 142 mm (1991; Table 3). Body size at metamorphosis varied from 31.6 to 46.6 mm SVL in *A. opacum*; two *A. talpoideum* cohorts were larger in body size, accumulated higher lipid reserves, and metamorphosed later than any *A. opacum* cohorts (Table 3).

Using data from 13 cohorts of *Ambystoma opacum* for which we had estimates of all variables, we observed a significant relationship between TL and SVL at metamorphosis ($F_{(1,11)} = 40.7$, $P < 0.001$, $R^2 = 0.78$); residuals output from this analysis served as a measure of size-adjusted lipid levels. In the full regression model for *A. opacum* ($F_{(3,9)} = 6.4$, $P < 0.05$, $R^2 = 0.68$), adult survival was related to size-adjusted lipids ($t = 2.70$, $P < 0.05$), weakly related to postmetamorphic rainfall ($t = 1.86$, $P < 0.10$), and unrelated to SVL ($t = 0.28$, $P > 0.70$). Model results for mean age at first reproduction were weaker ($F_{(3,9)} = 2.7$, $P < 0.10$, $R^2 = 0.47$), but adult age was negatively related to SVL at metamorphosis ($t = –2.71$, $P < 0.05$; Fig. 4), and unrelated to postmetamorphic rainfall ($t = 0.76$, $P > 0.40$) and size-adjusted lipids ($t = –0.89$, $P > 0.40$). Because we had only three cohorts with complete data for *A. talpoideum* we could not use a fully parameterized regression model, but general patterns appeared similar (Figs. 3, 4). Larger (and fatter) *A. talpoideum* metamorphs appeared to have higher survival ($F_{(1,1)} = 54.35$, $P < 0.10$, $R^2 = 0.98$), but relationships of metamorph traits to age were nonsignificant (although R^2 values were greater than 0.57).

Effect of lipids on survival

Regressions of TL and survival on SVL for *A. opacum* produced size-adjusted residuals of each variable; i.e., a measure of how fat a cohort was for its body size and a

Table 2 Summary of Pearson product–moment correlations among variables used in analyses of relationships between premetamorphic and postmetamorphic variables for *Ambystoma opacum*

Variable	SVL	Lipids	Rain	L density	Hydro	$S_{1–4}$	Age	Lip res	$S_{1–4}$ res
SVL	1.0 (26)	0.903^{a} (16)	0.221 (21)	–0.679^{a} (23)	0.456^{b} (26)	0.345 (14)	–0.644^{b} (13)	–0.001 (16)	0.001 (14)
Lipids		1.0 (16)	0.456 (13)	–0.817^{a} (15)	0.115 (16)	0.529^{c} (13)	–0.642^{b} (13)	0.429^{c} (16)	0.256 (13)
Rain			1.0 (21)	–0.043 (19)	–0.157 (21)	0.691^{a} (14)	–0.100 (13)	0.413 (13)	0.577^{b} (14)
L density				1.0 (23)	–0.164 (23)	–0.321 (13)	0.619^{b} (12)	–0.202 (15)	–0.099 (13)
Hydro					1.0 (26)	0.156 (14)	–0.143 (13)	–0.273 (16)	0.046 (14)
$S_{1–4}$						1.0 (14)	–0.138 (13)	0.729^{a} (13)	0.938^{a} (14)
Age							1.0 (13)	–0.204 (13)	0.066 (13)
Lip res								1.0 (16)	0.719^{a} (13)
$S_{1–4}$ res									1.0 (14)

Number of cohorts used is presented in parentheses

^{a} $P < 0.01$, ^{b} $P < 0.05$, ^{c} $P < 0.10$

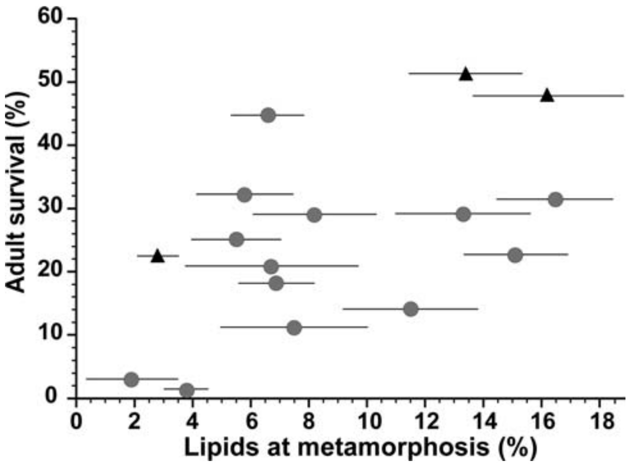

Fig. 3 Relationship between percent lipids at metamorphosis and percent survival (through age 4) of adults of *Ambystoma opacum* (*filled gray circles*) and *A. talpoideum* (*filled triangles*). Data points represent 16 separate cohorts (mean of 2,138 marked metamorphs per cohort, range 168–6,084 individuals) from two wetland breeding sites in seven years. *Horizontal bars* depict the standard deviation in percent lipids for each cohort (N = 6–62 individuals)

separate index of how well it survived for its size. A regression using these two sets of residuals explicitly tested for a connection between lipid reserves and adult survivorship independent of structural body size (i.e., SVL). Size-adjusted lipids explained 52% of the variance in size-adjusted survival ($F_{(1,11)} = 11.8$, $P < 0.01$; Fig. 5).

Discussion

Lipid reserves at metamorphosis were strongly related to body size across a wide range of larval environments for three amphibian species. In amphibians, the larval environment influences postmetamorphic performance (Altwegg 2003; Altwegg and Reyer 2003; Berven 1990; Goater 1994; Morey and Reznick 2001; Pechmann 1994; Pfennig et al. 1991; Scott 1994; Semlitsch et al. 1988; Smith 1987). Indeed, the larval environment—including food quantity and quality, intra- and interspecific larval densities, timing of pond filling and drying, temperature, predator presence or absence, species composition and densities—affects the survival and success of later-stage individuals in several taxa, including fish, freshwater and marine invertebrates, insects, and amphibians (reviewed by Pechenik et al. 1998), often by promoting body size variation at metamorphosis. In this study we observed that salamander larval density and pond hydroperiod covaried with size-related traits at metamorphosis, and these metamorph traits subsequently influenced adult survival to and age at first reproduction. Interestingly, whereas lipid stores acquired in the larval environment were related to adult survival, it was larval body size (i.e., SVL) that was related to age at first reproduction. A trade-off between larval allocation to fat stores or structural growth may determine whether an individual has a higher probability of survival (but reproduces later) or breeds at a younger age (if it survives).

Lipids and survival

Smaller size at metamorphosis often leads to lower postmetamorphic survival (Berven 1990; Goater 1994; Pechmann 1994; Relyea and Hoverman 2003; Scott 1994), but the mechanisms of this effect are poorly understood (Rothermel 2003). Large juvenile size may decrease susceptibility to starvation (Scott 1994), enhance locomotor performance (Álvarez and Nicieza 2002), reduce water loss (Spight 1968), lower predation risk (Berven 1990), and lessen chance of parasitic infection (Goater 1994). We observed that lipid reserves were strongly correlated with body size at metamorphosis in three amphibian species.

Table 3 Yearly variation metamorph and adult traits of *Ambystoma opacum* and *A. talpoideum* at two breeding sites on the Savannah River Site in South Carolina

Species	Year	Site	Type	SVL (mm)	Date (month/day)	Rain total (mm)	Total lipids (%)	Total marked (N_M)	Proportion surviving (S_{1-4})	Mean age (years)
A. opacum	1986	GB	Low	37.8	May 8	75.3	6.6	248	0.452	2.61
	1986	GB	High	31.6	May 8	75.3	5.5	168	0.250	2.71
	1986	RB	Bay	35.7	April 21	8.8	3.8	1100	0.011	2.58
	1987	GB	Low	41.6	May 13	42.8	7.5	868	0.111	2.40
	1987	GB	High	34.6	May 22	23.2	1.9	1360	0.030	3.02
	1989	GB	Bay	46.6	May 2	75.9	11.5	1898	0.143	2.10
	1990	GB	Bay	38.8	April 29	61.1	6.7	5072	0.195	3.10
	1991	GB	Bay	39.5	April 23	137.3	8.2	360	0.244	2.55
	1991	RB	Bay	45.5	April 20	141.8	16.5	375	0.306	2.60
	1993	GB	Bay	37.0	May 14	49.1	5.8	753	0.352	2.65
	1993	RB	Bay	46.3	May 5	31.4	15.1	5673	0.238	2.08
	1994	GB	Bay	39.6	May 4	22.3	6.9	1891	0.120	2.90
	1994	RB	Bay	42.5	May 4	22.3	13.3	6084	0.289	2.53
A. talpoideum	1987	RB	Bay	37.0	June 5	107.0	2.8	2314	0.237	2.57
	1991	RB	Bay	49.5	June 21	118.4	16.4	4051	0.478	1.99
	1991	GB	Bay	–	–	–	16.9	–	–	–
	1993	RB	Bay	49.1	June 11	85.5	13.4	802	0.512	1.56
	1993	GB	Bay	–	–	–	23.8	–	–	–
	1994	GB	Bay	–	–	–	11.3	–	–	–

Date is the cohort median date at metamorphosis; *Rain* represents the three-week cumulative rainfall post-metamorphosis; *Type* indicates animals that came from experimental manipulations of larval densities (Low or High, reported in Scott 1994) or from natural wetland populations. Animals were toe-clipped at metamorphosis and recaptured in subsequent years as breeding adults

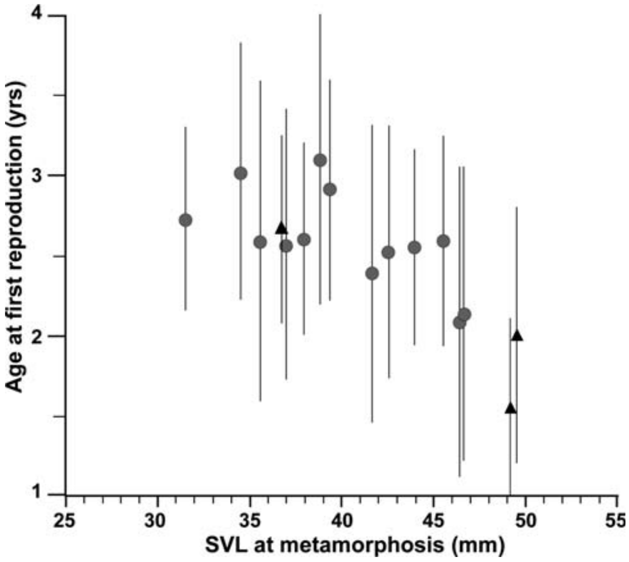

Fig. 4 Relationship between mean SVL at metamorphosis and mean age at first reproduction of *Ambystoma opacum* (*filled gray circles*) and *A. talpoideum* (*filled triangles*). Data points represent means ±1 SD for 16 separate cohorts from two wetland breeding sites in seven years

Salamander cohorts that were relatively fat for their body size also had comparatively high survival to first reproduction. In conjunction with a weak influence of cumulative postmetamorphic rainfall in the weeks following emergence from the pond, we propose that energy stores acquired in the larval stage are an important contributor to postmetamorphic success and, ultimately, fitness.

In amphibians, postmetamorphic terrestrial mortality appears to be highest immediately after metamorphosis (e.g., Altwegg and Reyer 2003; Creusere and Whitford 1976; Morey and Reznick 2001; Pfennig et al. 1991). In *A. opacum* and the spotted salamander, *A. maculatum*, the highest mortality occurs in the first summer after metamorphosis, with lower body mass individuals having lower survival than heavier animals (Rothermel and Semlitsch 2006). High mortality within a few months post-metamorphosis also occurs in the California tiger salamander, *A. californiense* (Trenham et al. 2000). In the reed frog (*Hyperolius viridiflavus*), postmetamorphic survival is likely determined by both water loss rates and energy stores, with small metamorphs allocating most of their assimilated energy to growth (to avoid dehydration), and switching allocation to lipid storage once a critical size is reached (to avoid starvation; Geise and Linsenmair 1988).

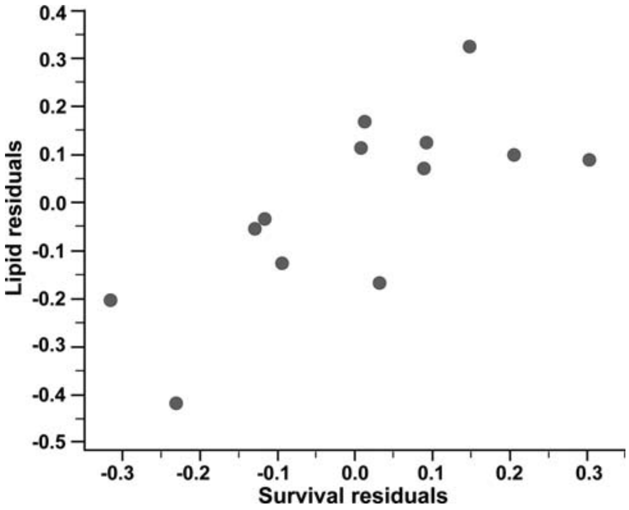

Fig. 5 Relationship between size-adjusted survival through age 4 (survival residuals) and size-adjusted lipids levels at metamorphosis (lipid residuals) of adult *Ambystoma opacum*. Data points represent 13 separate cohorts from two wetland breeding sites in seven years

Adult salamanders use fat body lipid stores during periods of prolonged starvation (Mould and Sever 1982), and in general larger amphibians are more resistant to starvation (Lilywhite et al. 1973; Pfennig et al. 1991). Our study demonstrates that stored lipids accord a similar buffer against environmental uncertainty in recently metamorphosed animals. Terrestrial salamanders often experience rainless periods with reduced feeding opportunities (Jaeger 1980). The threat of desiccation forces animals to retreat under surface objects or into burrows with higher moisture levels (Rothermel and Luhring 2005), but where food is also scarce (Jaeger 1978). Dehydration itself may limit food intake in amphibians (Larsen 1992), and the resulting starvation also reduces resting metabolic rate (Pinder et al. 1992). Thus, in the extreme situation of severely reduced feeding opportunities due to dry conditions, large animals have a better chance of survival due to disproportionately larger fat stores and lower metabolic rates (Pinder et al. 1992).

Species differences in allocation

All three species that we examined showed a similar relationship of total lipids to body size (Fig. 1). Lipid allocation to compartments differed between the two salamander species, although due to our limited data for *A. talpoideum* it is unclear whether this pattern reflects a true species difference or is primarily related the propensity for large metamorphs (regardless of species) to reproduce earlier. In previous studies, *A. opacum* and *A. talpoideum* have exhibited apparent differences in survival and mean age at maturity, with 67–85% of surviving *A. talpoideum* returning to breed at one year of age (4–6 months after metamorphosis), and 16–48% of the metamorphs surviving to reproduce at least once (Semlitsch et al. 1988; Pechmann 1995). In *A. opacum*, mean age at first reproduction is often older (2.5–4 years) and survival to first reproduction lower (Scott 1994) than *A. talpoideum*, even in the same habitat (Pechmann 1995). At first glance it appears that these two species differ significantly in two fitness traits. However, in both species adult survival and mean age at first reproduction are related to metamorph traits (Figs. 3, 4), with fatter metamorphs surviving better and larger individuals returning to breed at younger ages in both species. Previous studies have examined *A. talpoideum* cohorts that were of relatively large body size at metamorphosis (SVL 48.8–49.9 mm; Semlitsch et al. 1988; Pechmann 1995) and *A. opacum* that were relatively small (SVL 31.6–41.6; Pechmann 1995; Scott 1990). When compared at a common body size, adult survival to and mean age at first reproduction in these two species appear to be more similar.

We suggest that the ‘‘species difference’’ we observed in relative allocation of lipids to fat bodies is indicative of a size threshold, irrespective of species, at which an individual maximizes allocation to fat bodies as it increases lipid allocation to other (carcass) tissues. We assumed a linear relationship between lipids in all compartments and body size, and we used linear models to test these relationships. But there may be a switch point at approximately 0.42 g LDM (or roughly 42–46 mm SVL) when lipids are increasingly allocated to other organs; i.e., the relationship is nonlinear. In cohorts of natural and experimental enclosure *A. talpoideum* (DES unpublished data; Pechmann 1994), salamanders that return to breed 4–6 months following metamorphosis have often lost mass and gained little in SVL, suggesting that they fed little and mobilized stored lipids for maintenance and reproductive maturation. Increased lipid levels outside of the fat bodies—what we term carcass lipids, that includes liver and gonads—may indicate that allocation to gonadal development begins at metamorphosis in order to allow an animal to return in the first breeding season a few months later. There is evidence in fish species that control of sexual maturation is linked to ‘‘surplus’’ lipid reserve levels (Jonsson and Jonsson 2003), with a threshold level of fat needed to proceed with maturation (Simpson 1992). Alternatively, the lipid allocation patterns may reflect true species differences; further work examining differential lipid storage is needed to address these questions.

Trade-offs and amphibian models

Energy allocation patterns are central to understanding life-history decisions and trade-offs. Lipid reserves at one life stage affect later stages, whether it is egg provisions that influence juvenile performance (i.e., the offspring size vs.

clutch size trade-off; Emlet and Hoegh-Guldberg 1997), or energy stores acquired as larvae that affect adult traits, as observed in butterflies (Boggs 1986), fish (Ludsin and DeVries 1997), mussels (Philips 2004), barnacles (Hentschel and Emlet 2000), and other organisms.

Natural selection should favor the energy partitioning that maximizes fitness. Dynamic optimization models of energy allocation (see review by Perrin and Sibly 1993) identify when energy storage is adaptive, and generally predict allocation to growth early in a season with a later switch to storage. In juvenile fish, individuals allocate relatively more energy to growth and less to storage early, and increasingly more to storage as they get larger (Biro et al. 2005; Sogard and Spencer 2004). In amphibian larvae, energy allocation to early growth to reduce predation risk and achieve the minimum size necessary to metamorphose is paramount (Anholt et al. 2000), but as demonstrated here allocation to storage is also critical. Lipid accumulation during the larval period is related to factors such as larval density and pond permanency (this study; Pfennig 1992), and may be modulated by variation in the length of the larval period and developmental rate (Álvarez and Nicieza 2002) as well as other factors.

Many amphibians are pond-breeding species that have a complex life cycle (CLC; Wilbur 1980), with important trade-offs between age and size at metamorphosis and age and size at first reproduction. As in other CLC species (e.g., barnacles; Hentschel and Emlet 2000), it is essential to understand the energy partitioning in amphibians that underlies the switch from aquatic to terrestrial habitat, and the decision about when to mature. The larval period is certainly a period of development and growth (Wilbur and Collins 1973), but it is also a period during which lipids can be stored for later use. Amphibian larvae need lipid reserves during metamorphosis itself, a period when larvae do not feed due to changes in oral and digestive morphology (Beck and Congdon 2003). But amphibians do not exhaust energy stores during metamorphic climax, and emerge with size-dependent energy reserves (Beck and Congdon 2003, this study). It has been noted that individuals metamorphosing at a larger SVL (and its covariates) have numerous advantages, such as higher survival, earlier maturity, larger size at first reproduction and greater clutch size in females (Berven 1990; Scott 1994; Semlitsch et al. 1988; Smith 1987). If it were solely large SVL at metamorphosis that benefited adults, one might expect larvae to allocate minimal non-maintenance energy to storage (merely to meet the energy needs of metamorphosis) and most to somatic growth and maturation (Bernardo 1994). Our observations seem to indicate strong postmetamorphic selection to acquire ‘‘surplus’’ lipid reserves in the larval environment. Such selection pressure has been demonstrated for *Drosophila melanogaster*, in which selection for starvation resistance in the postmetamorphic environment shapes the evolution of larval allocation to fat reserves (Chippendale et al. 1996). Selection in water frogs (*R. lessonae* and *R. esculenta*) also favors large size at metamorphosis (Altwegg and Reyer 2003), possibly acting through correlated traits such as lipid stores.

We conducted a multiyear observational study in which the cohort-level relationships among larval environment, metamorph traits, and adult traits related to fitness suggest several important life-history trade-offs. A true demonstration of these trade-offs requires individual-level experimental manipulation of factors in the larval environment that promote differential larval investment in growth versus storage (such as food ration, food level variability, temperature, predation risk) and that will likely affect covariance between metamorph lipid level and body size and subsequent adult performance. Both temperature and diet influenced metamorph traits in Iberian painted frogs (*Discoglossus galganoi*), but lipid reserves seemed to be most dependent upon larval developmental rate and were not related to body size (Álvarez and Nicieza 2002), unlike our observations for three populations of *R. sphenocephala*. Audo et al. (1995) deprived *Hyla chrysoscelis* tadpoles of food at different stages and found that metamorphs from all treatments were equivalent energetically, which differs from the increased proportional allocation to lipids in larger animals we documented across a variety of larval habitats. In other taxa, Philips (2004) manipulated food level and temperature in mussels, generated larvae with relatively high lipids for their size and the converse, and examined postmetamorphic success. Biro et al. (2005) controlled food levels in whole-pond populations of young-of-the-year fish, sampled throughout early growth, and identified key switch points in energy allocation that were related to behavioral changes and subsequent survival. Although lipid levels at metamorphosis are important to postmetamorphic traits, these experimental studies demonstrate that other factors also contribute to adult success. Additional studies that explicitly identify how variability in the larval environment influences energy allocation, larval traits, and adult performance are needed.

Many conceptual models that focus on the timing of and size at metamorphosis emphasize the evolutionary trade-offs between larval growth and development, but generally do not address energy allocation to lipid storage (Alford and Harris 1988; Smith-Gill and Berven 1979; Leips and Travis 1994; Wilbur and Collins 1973). The implications of lipid storage and use should not be overlooked in the life history of amphibians that undergo seasonal energy deficits, whether as recent metamorphs, juveniles or adults (Bernardo 1994; Hom 1988). More explicit formulation of how postmetamorphic selection in terrestrial habitat (sensu Werner 1986; Rowe and Ludwig 1991; Day and Rowe 2002) molds premetamorphic energy allocation would lead to a better understanding of CLC reaction norms, as well as how larval quality influences population dynamics.

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