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Carry-over Effects of Size at Metamorphosis in Red-eyed Treefrogs: Higher Survival but Slower Growth of Larger Metamorphs

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ABSTRACT

Most animals have complex life histories, composed of a series of ecologically distinct stages, and the transitions between stages are often plastic. Anurans are models for research on complex life cycles. Many species exhibit plastic timing of and size at metamorphosis, due to both environmental constraints on larval growth and development and adaptive plastic responses to environmental variation. Models predicting optimal timing of metamorphosis balance cost/benefit ratios across stages, assuming that size affects growth and mortality rates in each stage. Much research has documented such effects in the larval period, but we lack an equal understanding of juvenile growth and mortality. Here, we examine how variation in size at metamorphosis in the Neotropical red-eyed treefrog, *Agalychnis callidryas,* affects post-metamorphic growth, foraging, and behavior in the lab as well as growth and survival in the field. Surprisingly, many individuals lost mass for weeks after metamorphosis. In the lab, larger metamorphs lost more mass following metamorphosis, ate similar amounts, had lower food conversion efficiencies, and grew more slowly after mass loss ceased than did smaller ones. In field cages larger metamorphs were more likely to survive than smaller ones; just one froglet died in the lab. Our data suggest that size-specific differences in physiology and behavior influence these trends. Comparing across species and studies, large size at metamorphosis generally confers higher survival; size effects on growth rates vary substantially among species, in both magnitude and direction, but may be stronger in the tropics.

Abstract in Spanish is available in the online version of this article.

Key words: *Agalychnis callidryas*; complex life cycle; foraging; juvenile behavior; optimal metamorphic timing; Panama; phenotypic plasticity; trade-off.

Complex life histories allow organisms to take advantage of resources in different environments at different life stages. Plasticity in the timing of transitions between stages permits organisms to balance costs and benefits across environments. For example, insects can alter the timing of metamorphosis in response to predator cues (Benard 2004), and some marine invertebrates delay metamorphosis when settlement substrate is unavailable (Pechenik 1999). Anurans exhibit plasticity in metamorphic timing in response to larval competition (Relyea & Hoverman 2003, Loman 2004, Boone 2005), larval and metamorph predation risk (Vonesh 2005, Vonesh & Bolker 2005, Vonesh & Warkentin 2006), and pond drying (Semlitsch *et al.* 1988, Laurila & Kujasalo 1999, Leips *et al.* 2000). Metamorphic timing and metamorph size also reflect genetic variation (Travis *et al.* 1987, Newman 1988, Briggs 2013) and constraints of the larval environment (*e.g.*, low temperature or food) since larvae must reach some minimum size before they can metamorphose (Goater 1994, Morey & Reznick 2001, Relyea & Hoverman 2003, Capellán & Nicieza 2007, Stamper *et al.* 2008).

Models for the optimal timing of metamorphosis have been based on minimizing the ratio of mortality rate (μ) to growth rate (g), which maximizes the chance of reaching reproductive size; metamorphosis is predicted to occur when μ/g becomes lower in the next environment than in the current one (Werner & Gilliam 1984, Werner 1986). For example, a slow-growing tadpole is predicted to metamorphose at a smaller size than a rapidly growing tadpole to capitalize on the possibility of faster growth in the next life stage (Werner 1986). These small metamorphs may improve their growth but are more likely to be consumed by juvenile stage predators because their size compromises locomotor performance (Wilbur 1980, Chelgren *et al.* 2006, Ficetola & De Bernardi 2006). Trade-off patterns and growth strategies may be fundamentally different across environments that vary in seasonality and resource availability or among species that share environments but differ substantially in natural history.

Plastic metamorphic timing results in a range of sizes at metamorphosis. Theory and some empirical data suggest that larger froglets grow faster (Werner 1986, Altwegg & Reyer 2003)

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and reach a larger size at reproductive age (Berven 1982, Altwegg & Reyer 2003), resulting in higher fecundity for females (Berven 1988, Camargo *et al.* 2008), a competitive advantage for males (Wells 1977, Wilbur *et al.* 1978, Berven 1981, Briggs 2008), and greater survival on average (Goater 1994, Morey & Reznick 2001, Altwegg & Reyer 2003, Chelgren *et al.* 2006). Reduced larval density consistently produces larger metamorphs; however, the effect of larval density on post-metamorphic growth varies considerably (*e.g.,* none: Morey & Reznick 2001, negative: Gramapurohit 2009, positive: Goater 1994). Hence, although large size at metamorphosis is considered advantageous, neither size nor larval density seems sufficient to explain the observed variation in post-metamorphic growth rate across studies. The long-term effects of size at metamorphosis on anuran survival and reproductive success, and the mechanisms that drive them, may vary more than is currently appreciated.

Although we understand much about how larval environments affect anuran size at metamorphosis (Wilbur 1980, Vonesh & Bolker 2005, Relyea 2007, Touchon *et al.* 2013a), we know relatively little about how variation in size at metamorphosis affects the juvenile life stage, especially in the tropics where most anuran diversity occurs (Duellman 1999). Only four studies of post-metamorphic growth have been conducted with tropical species (Hu *et al.* 2008, Gramapurohit 2009, Van Allen *et al.* 2010, Cabrera-Guzman *et al.* 2013), compared to 10 of temperate species (see discussion). Life histories of tropical and temperate anurans often differ substantially (Morrison & Hero 2003), and we know almost nothing about metamorph behavior and growth in the wild anywhere (Relyea 2007).

Neotropical red-eyed treefrogs (*Agalychnis callidryas*) exhibit substantial plasticity in metamorphosis, including three-fold variation in mass at tail resorption (Bouchard *et al.* 2011, Touchon *et al.* 2013a). This variation facilitates tests for carryover effects of metamorph size (Touchon *et al.* 2013b). Here, we generate a range of sizes of *A. callidryas* metamorphs by varying larval density in mesocosms. We then assess effects of size at metamorphosis on post-metamorphic growth, feeding rates, and activity of *A. callidryas* froglets raised individually in the lab and determine the effects of metamorph size on survival and growth of *A. callidryas* froglets raised individually in the field. We discuss our results in the context of other studies of post-metamorphic growth and survival to highlight potential patterns across latitudes, species, and study design.

METHODS

Larval rearing.—Research was conducted in 2009 at the Smithsonian Tropical Research Institute, Gamboa, Panama. *Agalychnis callidryas* froglets were obtained from a larval mesocosm experiment. Briefly, fifty *A. callidryas* egg clutches from local ponds hatched at the same age (6 d) were pooled and distributed at densities of 5, 25, and 50 tadpoles in 400-L mesocosms. Emerged metamorphs were collected daily and held in plastic cups to complete tail resorption. We use mass at tail resorption (stage 46, Gosner 1960) as initial froglet mass. To minimize variation in larval period length, we used animals that finished resorbing their tails between 28 and 30 June. Thus, our sample includes animals that emerged relatively early from the high-density tanks, during the shorter emergence period of tadpoles from medium- and low-density tanks. From these, we selected 28 that were evenly distributed across the range of metamorph mass (0.20–1.02 g) and snout-vent length (SVL 15.8–22.5 mm, measured with calipers). To assess tadpole morphology, we photographed two-thirds of the tadpoles from each tank a week before metamorphosis began. Tadpole body and tail length were measured using ImageJ (Rasband 2012).

Lab froglet rearing.—Froglets were reared individually in 36 × 22 × 23 cm plastic terraria containing a stick, live vegetation, a cup of water, and a Petri dish with a slice of banana for fruit flies. Terraria were assigned to randomized locations on shelving in an ambient temperature and humidity room with lights on 0600 h to 1800 h. Cages were cleaned weekly and sprayed twice daily with aged tap water to maintain high humidity. Froglet mass and SVL were measured every 7 d for 6 weeks, then frogs were released near their pond of origin.

We attempted to provide insect prey *ad libitum* while limiting disturbance of terraria, erring on the side of more frequent feeding if in doubt. Initially, we supplied froglets with locally collected worker termites (Isoptera) and fruit flies (Van Allen *et al.* 2010). Because termites survived poorly in terraria, after 18 d we switched to sweep-netted insects, mostly leafhoppers (Cicadellidae), plus fruit flies. Each froglet was given 134 ± 23 fruit flies and 120 ± 14 leafhoppers per feeding (estimated from $N = 9$, 14 samples, respectively; mean ± SE, here and throughout unless noted). Insects were replenished every time we opened a terrarium to clean it or measure the frog or if the number of active insects visible was reduced, based on daily checks (≤20 insects easily seen; more were present but obscured by vegetation). Initial feeding intervals were 2.86 ± 0.11 d (range 1–7 d). Starting at 23 d, when we began collecting feces to analyze intake, frogs were fed at least every 3 d (1.57 ± 0.03 d, 1–3 d). Flies were dusted with Reptocal™ (Tetrafauna™) vitamins weekly to improve nutrition (as is common in anuran husbandry, Pough 2007).

Field froglet rearing.—We also raised metamorphs of a range of sizes in the field. Most were from the same mesocosm experiment as our lab-reared frogs; some were taken from an experiment manipulating resource levels and predation risk (Touchon *et al.* 2013a) after metamorphs ceased emerging from the first mesocosm experiment. Frogs were placed in tubular mesh cages in shady areas of the forest near the Experimental Pond (9°7′14.88″N, 79°42′14.11″W). Cages (80 × 50 cm, 2–5 mm diameter holes to retain froglets but allow passage of insect prey) enclosed sections of tree branches at 1–2 m height and were tied closed at both ends. Each frog was provided with a moist refuge, a cup of water, and a banana slice to attract flies. When it did not rain, we misted cages twice daily to maintain humidity. Mass and SVL were measured at 2 and 4 weeks. We

measured frogs less frequently than in the lab because it involved greater disturbance to the frog and within-cage habitat. Sixty-nine froglets were placed in cages but 37 individuals went missing when their cages were damaged. To assess effects of initial size on mortality, we exclude all potential escapees and compare sizes of animals known to have died or survived. To assess effects on growth, we analyze the relationship of initial size and growth for 14 froglets that survived for 4 weeks. We did not measure activity or feeding behavior of field-reared froglets.

Food intake analysis.—We removed all feces from laboratory cages at ~23 d after tail resorption, then collected all feces every 3 d and preserved them in alcohol. Insect heads in each sample containing 3 d of feces were counted under a dissecting microscope. The width of fly heads and length of leafhopper heads (up to 30 per sample) were measured using an ocular micrometer. As nearly all insects consumed were flies and leafhoppers, we calculated mean daily intake (insects per day) from the number of fly and leafhopper head capsules in 3-d fecal samples, averaged across all samples for a frog. To compare dietary selectivity for prey size and type, for each individual we calculated average and maximum prey size and the proportion of intake that was flies, and regressed these against initial froglet size. To assess size effects on growth per unit intake (conversion efficiency), we divided average growth rates over 3 weeks (21–42 d) by average number of insects consumed per day, measured during the same period (~23–42 d).

Activity analysis.—Each laboratory-raised frog was videotaped for one night from *ca* 1730 to 0800 h, using the time-lapse function of a Sony DCR-TRV280 digital video camera to record 2 of every 30 sec. Frogs were randomly assigned to videotaping dates from 27 to 52 d; we had more frogs than nights during the measured growth period, necessitating the extended time. Frogs were recorded in their home containers under infrared and dim red illumination. Adult *A. callidryas* behave normally under such illumination (Caldwell *et al.* 2010). We analyzed videos with JWatcher (Blumstein *et al.* 2006), noting behavior in each 2-sec sequence and inferring behavior during the previous 28 sec. Behavioral categories were: walked, jumped, lunged (attempted prey capture), moved (walking or jumping indistinguishable), sat alert (eyes open, upright posture, still), turned head, changed posture, slept, defecated, and not visible. We used this information to reconstruct each frog's activity pattern from waking to returning to sleep. For each frog, we calculated activity period as the total time the frog was awake and visible, and activity level as the proportion of the activity period spent moving (walking, jumping, lunging, and moving).

Statistics.—We used R v.2.15.2 (R Development Core Team 2011) for all analyses. For multivariate models, we used a reverse stepwise-regression model selection approach with AIC as the criterion. Non-significant predictors were removed sequentially according to their P values in the model; the less-parameterized model was kept if a likelihood ratio test showed the models not to be significantly different. In some cases, a non-significant predictor remained. For relationships between two normally distributed numerical variables, we used linear regression. For non-normal data, we used Spearman's rank correlation.

Initial inputs to the model predicting 6-week post-metamorphic growth rates for lab frogs were initial mass (at tail resorption), larval density, duration of the mass-loss period (see below), proportion of initial mass lost during the mass-loss period, cumulative feeding delay (see below), a proportion of initial mass lost during the mass-loss period by cumulative feeding delay interaction, activity level, activity period, average intake, an initial mass by average intake interaction, and interactions between average intake and both measures of activity. We used the same initial inputs to predict growth rate following the initial mass-loss period.

We were more limited in our analysis of froglet growth in the field. We used a generalized linear model with a binomial distribution to test if initial mass or mesocosm experiment predicted survival to 4 weeks. Then, because the mass distribution for froglets that died was non-normal, we used a Wilcoxon rank-sum test to determine if initial mass differed between frogs that survived to 4 weeks and frogs that died (N = 14, 18, respectively), excluding all froglets whose fate was unknown. Mortality of lab-reared froglets was too low to test for size effects.

We considered the end of the post-metamorphic period of mass loss to be the first week with a mass gain or a loss <0.03 g, within the range of daily fluctuations (R. Tarvin & K. Warkentin pers. obs.). We used a generalized linear model with a binomial distribution to determine what best predicted whether a frog experienced mass loss before growth. Our initial inputs to this model were initial mass, cumulative feeding delay, and larval density. Mass-loss period durations were not normally distributed, and residuals from a generalized linear model were heteroscedastic, so we present only results of a Spearman's correlation.

To assess any potential effects of the greater variation in feeding intervals during the first 3 weeks, we calculated the cumulative feeding delay for each frog. We conservatively hypothesized that, even though there were abundant insects visible in terraria, intervals >3 d between insect replenishment might have constrained foraging. We calculated the feeding delay by subtracting 3 d from each feeding interval, then summing the remainders. We included feeding delay in stepwise-regression models to assess its contribution to mass loss and growth.

RESULTS

Larval and initial froglet traits.—Tadpoles from high-density tanks (50 tadpoles) had relatively longer head-body lengths and shorter tails than those from the low density (five tadpoles), but not the medium density (25 tadpoles) tanks ($F_{2,111}$ = 21.16, P < 0.0001 multiple R^2 = 0.28, adjusted R^2 = 0.26). Following tail resorption, the size of froglets to be reared in the lab and field (Table 1) encompassed much of the total variation in

froglets from the mesocosm experiment (mass: 0.19–1.05, 0.52 ± 0.17 g; SVL: 14.5–24.0, 19.3 ± 1.9 mm; range, mean ± SD). Average sizes of lab and field froglets were similar (Wilcoxon Rank-Sum test: $W = 1091$, $P = 0.32$; Table 1). There was no overlap in froglet mass between rearing densities in our sample; larger frogs came from lower density larval environments.

FROGLET GROWTH.—Over 6 weeks in the lab, frogs grew in SVL but decreased in mass (Fig. 1, Table 1). The best-fitting model of average post-metamorphic growth rate in the lab included initial mass, proportion of mass lost during the mass-loss period, and duration of the activity period ($F_{3,21} = 45.92$, $P < 0.0001$, multiple $R^2 = 0.87$, adjusted $R^2 = 0.85$; three individuals excluded due to lack of activity period data). Lab metamorphs that grew more slowly were larger at metamorphosis, lost proportionally more mass during the mass-loss period, and had shorter activity periods (Table 2). Overall, larger frogs grew less than did smaller frogs in the field (Fig. 2A: $F_{1,12} = 12.57$, $t = -3.55$, $P = 0.0040$, multiple $R^2 = 0.51$, adjusted $R^2 = 0.47$) and in the lab (Fig. 2B: $F_{1,25} = 74.9$, $t = -8.65$, $P < 0.0001$, multiple $R^2 = 0.75$, adjusted $R^2 = 0.74$). Analyses using SVL showed similar but weaker patterns. At 6 weeks after tail resorption, even though the post-metamorphic period of mass loss had ended for all lab frogs, 20 frogs still weighed less than at tail resorption. In the field experiment, only one of 14 surviving metamorphs gained mass over 4 weeks (Fig. 2A).

MASS LOSS.—More than half of the lab-reared frogs lost mass following metamorphosis for a period of 1–3 weeks. Individuals that lost mass were from lower density larval tanks ($\chi^2_{2,24} = 26.17$, $P = 0.040$) and were larger at metamorphosis ($\chi^2_{1,26} = 32.61$, $P = 0.018$). Larger metamorphs experienced longer mass-loss periods (Spearman's test: $S = 2011.48$, $P = 0.017$, $\rho = 0.45$) and lost more mass during that time (Spearman's test: $S = 1545.39$, $P = 0.0013$, $\rho = 0.58$; Figs. 3A and B). During the first 4 weeks, field froglets lost mass more quickly than lab froglets (two-tailed unpaired t-test: $t = 6.53$, df = 16.19, $P < 0.0001$). All but 2 of 27 individuals lost mass over the first 2 weeks, and only one of the remaining 14 frogs maintained its mass to 4 weeks.

Average growth rates in the lab after the mass-loss period were predicted by initial mass, proportion of mass lost, and activity period ($F_{3,21} = 15.25$, $P < 0.0001$, multiple $R^2 = 0.69$, adjusted $R^2 = 0.64$; three individuals excluded due to lack of activity period data; Fig. 3C, Table 2). Individuals that grew the fastest were smaller at metamorphosis, lost proportionally more mass during the mass-loss period and tended to have longer activity periods. Results were similar for an analysis restricted to the period after all individuals had ceased losing mass.

TABLE 1. *Sizes of* Agalychnis callidryas *froglets at tail resorption and after 6 weeks in the lab or 4 weeks in field cages.*

	Lab			Field		
	Range	Mean	SD	Range	Mean	SD
Initial mass (g)	0.20–1.02	0.58	0.21	0.20–0.92	0.53	0.18
Final mass (g)	0.37–0.80	0.53	0.12	0.20–0.70	0.40	0.15
Initial SVL (mm)	15.8–22.5	19.8	2.0	14.5–24.0	20.2	2.1
Final SVL (mm)	19.6–26.2	22.8	2.1	17.4–26.1	22.3	2.6

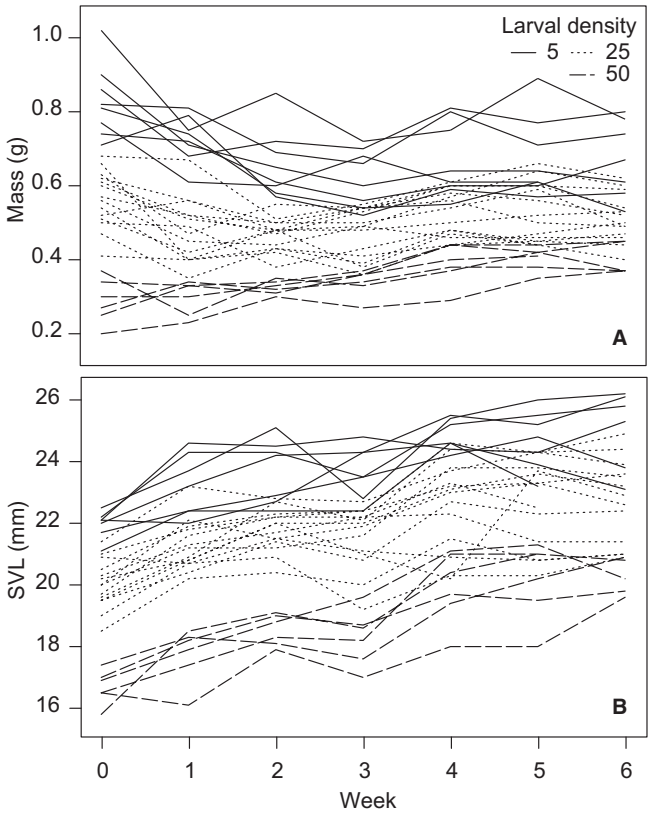

FIGURE 1. Smaller red-eyed treefrog (*Agalychnis callidryas*) metamorphs grew faster than did larger metamorphs in both (A) mass and (B) snout-vent length. Each line represents growth over 6 weeks for one lab-raised froglet. Tadpoles were raised at different densities to generate variation in size at metamorphosis.

MORTALITY.—In the lab, only one froglet died. Of 69 field-raised froglets, 37 individuals went missing when their cage was damaged; they probably escaped. Missing froglets and those with known fates did not differ in initial size (0.53 ± 0.15 g vs 0.54 ± 0.22 g; Wilcoxon rank-sum test: $W = 572$, $P = 0.81$). We restricted our analysis to the 32 individuals with known fates, of which 14 survived to 4 weeks and 18 died. Initial mass significantly predicted survival ($Z_{1,30} = 2.53$, $P = 0.011$); metamorphs that survived were larger than those that died (Wilcoxon rank-sum test: $W = 59.5$, $P = 0.012$). Survival was not related to the mesocosm experiment from which the froglet came ($Z_{1,30} = -0.91$, $P = 0.36$).

FOOD INTAKE.—The maximum number of insects (125) ingested by any frog during a 3-d period was much less than the number offered per feeding (~250 insects), and the average feeding rate

TABLE 2. *Predictors in models of Agalychnis callidryas froglet growth rates over the 6 weeks following tail resorption and over the weeks following the mass-loss period.*[a]

	Factor	Regression coefficient ± SE	*t*	*P*	*N*
Growth over 6 weeks	Initial mass	–0.076 ± 0.0086	–8.80	<0.0001	25
	Proportion mass lost during the mass-loss period	–0.032 ± 0.013	–2.44	0.023	25
	Duration of activity period	0.000027 ± 0.000011	2.44	0.024	25
Growth following the mass-loss period	Initial mass	–0.059 ± 0.010	–5.10	<0.0001	25
	Proportion mass lost during the mass-loss period	0.080 ± 0.016	5.10	<0.0001	25
	Duration of activity period	0.000025 ± 0.000013	1.86	0.078	25

[a]Growth period following mass loss ranged from 3 to 6 weeks, depending on the length of the mass-loss period for each individual.

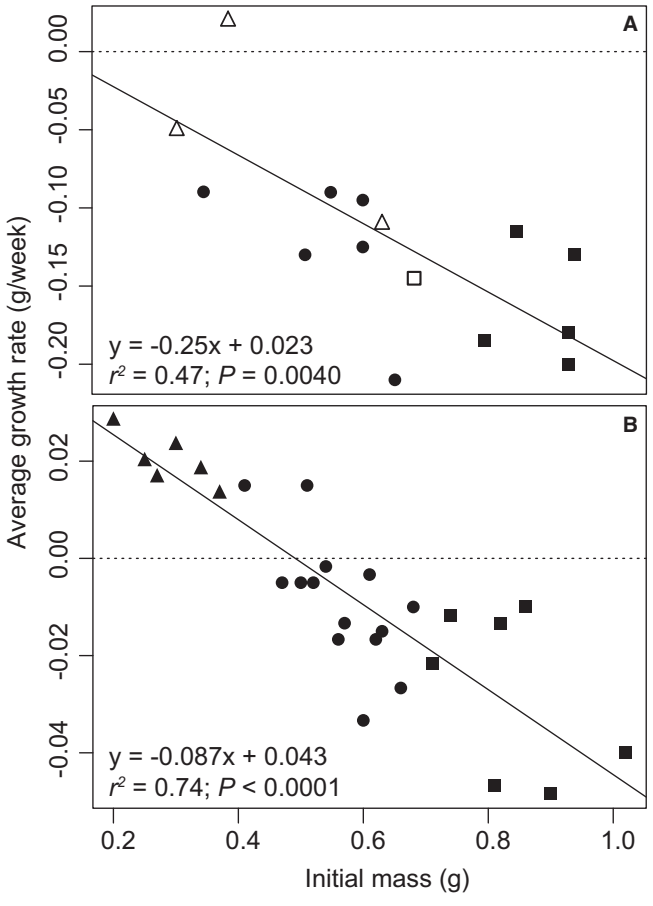

FIGURE 2. Average post-metamorphic growth rates for individual froglets (A) in the field (N = 14) and (B) in the lab (N = 27) as a function of mass at tail resorption. Tadpoles were raised at different larval densities (closed symbols: triangle = 50, circle = 25, square = 5 tadpoles/tank) or resource levels (open symbols: triangle = high, square = low) to generate size variation.

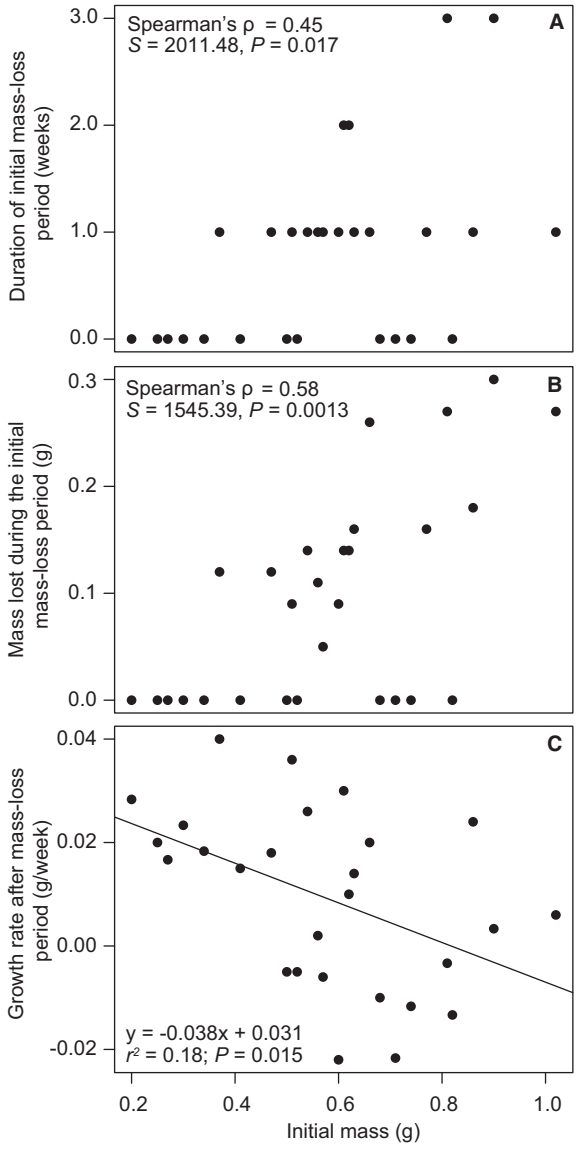

FIGURE 3. Relationship between froglet mass at tail resorption and (A) duration of the post-metamorphic mass-loss period (N = 28), (B) mass lost during that period (N = 28), and (C) average growth rate following the mass-loss period (N = 27; one individual died between week 5 and 6 and is not included).

was much lower (35 insects/period). During the first 3 weeks, before fecal collections, 24 of 28 lab-reared frogs experienced one or more intervals of >3 d between feedings, as insects in their terraria were not visibly depleted.

Average daily intake did not predict growth. We analyzed 435 frog-days of feces (145 3-d samples, 5.2 ± 0.3 per frog).

TABLE 3. *Correlations of the size of* Agalychnis callidryas *froglets at tail resorption with measures of foraging and activity in the post-metamorphic period. Statistics are from linear models of individual factors regressed against initial froglet mass. Foraging was measured between 23 and 42 d after tail resorption. Activity was measured through one randomly selected night during this period.*

Factor	F	t	P	N	Multiple R^2	Adjusted R^2
Largest prey item	7.19	2.68	0.013	28	0.22	0.19
Average prey size	0.22	0.47	0.65	28	0.0083	–0.030
Prop. of each prey type consumed	0.045	0.21	0.83	28	0.0017	–0.037
Intake (insects/day)	0.0065	–0.08	0.94	28	0.00025	–0.038
Activity level	0.00016	0.013	0.99	25	<0.0001	–0.043
Activity period	0.21	–0.45	0.65	25	0.0089	–0.034

Frogs ate 11.7 ± 0.7 insects (range 1–42) per day and consumed more flies than leafhoppers (7.8 ± 0.6 vs 3.9 ± 0.2). Leafhopper head capsules (1.05 ± 0.0058 mm, 0.29–2.37 mm, $N = 1614$) were on average larger than fly head capsules (0.71 ± 0.0028 mm, 0.30–2.00 mm, $N = 2329$). Initial froglet size was related to the size of the largest prey consumed, but not to average prey size, nor to proportions of each prey type consumed (Table 3). Larger metamorphs did not consume more insects per day (Table 3). Furthermore, for their intake level, larger froglets grew less than did smaller froglets (Spearman's test: $S = 5040$, $P = 0.047$, $\rho = -0.38$).

Behavior.—We captured the entire nocturnal activity period for most frogs; all but three were asleep at the start of recording, and all but five were asleep again before the end. Frogs typically awoke within 1.5 h after lights out (0.96 ± 0.44 h, $N = 10$) and often defecated soon thereafter. Most frogs fell asleep within 1 h after lights on (0.83 ± 0.30 h, $N = 15$). Activity patterns and behaviors were highly variable. Some frogs spent nearly the entire night walking along the terrarium walls, while others hardly moved. Although activity period duration was a predictor in our growth models, there was no evidence that activity level or period correlated with initial froglet size (Table 3), and neither measure of activity predicted average intake (activity level: $F_{1,23} = 0.00066$, $t = 0.026$, $P = 0.98$, multiple $R^2 < 0.0001$, adjusted $R^2 = -0.043$; activity period: $F_{1,23} = 0.0097$, $t = 0.099$, $P = 0.92$, multiple $R^2 < 0.00042$, adjusted $R^2 = -0.043$).

DISCUSSION

Many organisms with complex life cycles respond to environmental conditions by altering the timing of transition between life stages, resulting in a range of sizes at metamorphosis that has lasting effects on adult phenotypes and fitness. Many models of metamorphic timing build on a framework of minimizing the ratio of mortality to growth across stages (Werner 1986). These rates were initially conceived as simple functions of size (Werner & Gilliam 1984, Werner 1986), but studies of larval plasticity have revealed more complex patterns (Vonesh & Bolker 2005, Relyea 2007, Touchon *et al.* 2013a) and there is now a large body of research addressing larval mortality and growth. However, we know substantially less about how growth and mortality relate to size or environmental conditions following metamorphosis. We assessed effects of size at metamorphosis on patterns of growth, survival, feeding rates, and activity in the Neotropical red-eyed treefrog (*Agalychnis callidryas*). We found that initial mass positively influenced survival but negatively influenced post-metamorphic growth. Larger froglets lost more mass immediately following metamorphosis and then grew more slowly than small froglets. Additionally, larger metamorphs ate no more than small metamorphs, and activity was positively related to growth, suggesting that larger metamorphs might compromise growth to improve survival.

Agalychnis callidryas post-metamorphic growth rates and mass loss may be explained in part by variation in foraging behavior and digestive efficiency. Early in the juvenile period, larger metamorphs tend to eat relatively less than small metamorphs for their size (Morey & Reznick 2001, Hu *et al.* 2008, Benard & Maher 2011, Bouchard *et al.* 2011, this study); they also have larger fat stores than small individuals (Gramapurohit *et al.* 1998, Jenney *et al.* 2012). Because activity increases risk of predation as well as dehydration (Heatwole *et al.* 1969, Touchon *et al.* 2013b), decreased foraging activity could be a survival strategy available to large froglets (Werner & Anholt 1993, Scott *et al.* 2007). However, smaller metamorphs have greater digestive efficiencies and higher consumption rates (Hu *et al.* 2008, Van Allen *et al.* 2010, Benard & Maher 2011, Bouchard *et al.* 2011, Jenney *et al.* 2012), which together may improve growth rate and their chance of reaching reproductive size. Nevertheless, if smaller metamorphs cannot afford slowed growth, or undergo catch-up growth, they may often experience higher mortality (Rowe & Ludwig 1991).

Very little is known about the behavior or mortality of juvenile anurans in the wild, but the first few months following metamorphosis are likely to be a period of high risk (Wells 2007). Juvenile frogs generally suffer high mortality from predation and dehydration (Schmid 1965, Vonesh 2005), especially in the first few weeks after metamorphosis (Goater 1994, Altwegg & Reyer 2003, Chelgren *et al.* 2006, Harper & Semlitsch 2007). More than half of our field-raised frogs died within 4 weeks; this is not surprising given that some large lab-raised *A. callidryas* froglets lost mass equivalent to smaller froglets (~0.3 g).

The accessible cage locations we chose might not represent typical juvenile habitat, as their climbing behavior upon release (R. Tarvin & K. Warkentin pers. obs.) suggests juveniles may often be higher in trees. However, we believe that mortality was probably reduced in our field experiment because cages were kept moist, fruit cups attracted prey and, even though two frogs were eaten by ants, the mesh excluded some predators. Thus, natural mortality rates of red-eyed treefrog metamorphs could be higher than our estimates.

The lack of correlation between intake and growth or activity was unexpected, as were our observations of mass loss even with abundant prey available. The timing of measurements of activity and intake may have obscured patterns with growth; both were measured after feeding delays and mass-loss periods had ended so we were unable to test correlations with concurrent intake and activity. In addition, because we were only able to videotape one night per individual, our activity measurements do not capture any developmental variation in behavior. Mass loss is typical during metamorphosis while frogs cease foraging (Orizaola & Laurila 2009, Kuan & Lin 2011), but it appears to be less common following metamorphosis. Nevertheless, post-metamorphic mass loss has been

TABLE 4. *Effects of metamorph size (MS), or larval conditions that generate variation in MS, on post-metamorphic growth and survival in temperate and tropical anurans. For the period of juvenile growth measurement: I, froglets raised individually; G, froglets raised in groups; L, froglets raised in lab cages; O, froglets raised in outdoor enclosures; CRC, froglets were captured and recaptured in the field; +, positive correlation; –, negative correlation; (+) or (–), marginal correlation, 0.05 < P < 0.1; 0, no correlation; . . ., data not available.*

	Larval treatment		Juvenile measurement		Effect of MS		
Species	Manipulation (levels)	Effect on MS[a]	Rearing envt.	Period/intervals	Growth	Survival	Source
Temperate							
Bufo bufo	Density (2)	–	IL	28 d/14 d	+[b]	. . .	Goater *et al.* (1993)
Bufo bufo	Density (2)	–	IL/GL	126 d/21 d	+/0[b,c]	0/+[b,c]	Goater (1994)
Bufo woodhousii	Density (2)	–	GO	Overwinter	0	(+/−)c	Boone (2005)
Hyla versicolor	Density (2)	–	GL	29 d/29 d	0[b,d]	+[b]	Relyea and Hoverman (2003)
Pseudacris triseriata	Density (109)[e]	– (SVL)	CRC	2 yr/1 yr	. . .	+[f]	Smith (1987)
Pseudacris crucifer	Food (5)	0	IL	50 d/50 d	–	. . .	Van Allen *et al.* (2010)
Rana sphenocephala	Density (2)	–	GO	Overwinter	0	+	Boone (2005)
Rana blairi	Density (2)	–	GO	Overwinter	0	0	Boone (2005)
Rana clamitans	Density (2)	. . .[g]	GO	Overwinter	0	(+)	Boone (2005)
Rana sylvatica	Density (3)	–	GL	63 d/21 d	0[b]	0[b]	Goater and Vandenbos (1997)
Rana sylvatica	Food (3)	+	GO	29 d/29 d	0[d]	. . .	Benard and Maher (2011)
Rana temporaria	Density (2)	–	GL	56 d/14 d	. . . [h]	+[b]	Stamper *et al.* (2008)
Spea hammondii	Density (2)	–	IL	6 mo/1 mo	0	+	Morey and Reznick (2001)
			GO	6 mo/4 mo	0	+	
Tropical							
Agalychnis callidryas	Density (3)	–	IL	42 d/7 d	–	0	This study
			IO	28 d/14 d	–	+	
Agalychnis callidryas	Density (2)	–	IL	30 d/10 d	. . .[i]	0[b]	Van Allen *et al.* (2010)
Euphlyctis cyanophlyctis	Field collection	. . .	GO	1 yr/1 mo	–	0	Gramapurohit (2009)
Rhinella marina	Density (2)	–	GO	65 d/14 d	0	+	Cabrera-Guzmán *et al.* (2013)
	Density (2)	–	GO	185 d/14 d[j]	+	+	
	Density (3)	–	GO	40 d/14 d	+	0	
Xenopus laevis	Food (3)	+	IL	21 d/21 d	–	. . .	Hu *et al.* (2008)

[a]Mass unless otherwise noted.

[b]Effect of metamorph size inferred from tests of larval treatment effect.

[c]The effect of size varied across levels of another factor.

[d]Growth was measured for the group, not per individual.

[e]Measured natural variation across 109 pools.

[f]Higher survival to maturity via earlier maturation. No effect on survival to second breeding season.

[g]Unable to measure the effect of density since too few from the high density metamorphosed.

[h]Significant initial size differences disappeared by the end of the study.

[i]Frogs did not grow, so size effects on growth could not be determined.

[j]Until day 50, then only at days 134 and 185.

found previously in *A. callidryas* and also in *Rana sylvatica* (Van Allen *et al.* 2010, Benard & Maher 2011, Bouchard *et al.* 2011); whether this is widespread is unclear as other studies measured size at longer intervals (Table 4) and may not have detected a short mass-loss period. Nevertheless, we believe that feeding delays and mass loss in our study reflect delayed onset of foraging (Bouchard *et al.* 2011, Jenney *et al.* 2012) or low foraging activity and that mass loss was not a consequence of inadequate food availability. Future studies should investigate how intake and behavior vary over time following metamorphosis.

Studies of post-metamorphic growth in 14 anurans (four tropical, 10 temperate) do not show a consistent relationship between metamorph size and growth. Both positive and negative correlations are found in both temperate and tropical species, as well no correlation, which may be real or reflect insufficient power (Table 4). However, for the five species tested more than once, relationships between size and growth are largely consistent, and in two species this extends across lab and field studies, suggesting there may be species-specific variation in the effect of metamorph size on growth. Moreover, an effect of size at metamorphosis, whether positive or negative, appears to be stronger or more common in tropical than temperate anurans (significant correlations in 6 of 7 tests and 4 of 4 species vs. 3 of 13 tests and 2 of 10 species, Table 4). It is not clear, at this point, what causes this interspecific variation, or the potential latitudinal difference. They may be due to differences in natural history or environmental factors; however, the pattern might simply reflect the preponderance of studies of temperate *Rana,* which showed no relationship between metamorph size and growth (6 of 13 total temperate tests). Determining why some species show clear positive effects of metamorph size on growth, others show clear negative effects, and others undetectably weak or no effect, will require both greater attention to underlying mechanisms within species and a larger sampling of species across environments and life history diversity.

In contrast to the variation in effects on growth, across studies and latitudes, all clear effects of metamorph size on survival are positive (Table 4). In protected laboratory environments, a few studies found no size effect on survival. Under field conditions only one study (Gramapurohit 2009) found no evidence for a survival benefit of large size and another (Boone 2005) found a marginally significant interaction suggesting a cost of large size under some circumstances. Thus, large size at metamorphosis appears to be more consistently favored for enhancing survival than for any effect on growth. Metamorphosing at a larger size, with greater accumulated reserves, presumably lessens the immediate risk of starvation. It may also decrease the risk of both dehydration and predation because accumulated resources enable decreased activity.

Improving survival through a high-risk period after transitioning to the juvenile environment is likely a substantial benefit but may, in some cases, require compromising growth rates. Our results should motivate additional research into the mechanisms underlying variation in post-metamorphic growth, survival, and behavior, and how and when selective benefits accrue to larger metamorphs across environments and life histories.

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