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Putting μ/g in a new light: plasticity in life history switch points reflects fine-scale adaptive responses

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Abstract. Life history theory predicts that organisms with complex life cycles should transition between life stages when the ratio of growth rate (g) to risk of mortality (μ) in the current stage falls below that in the subsequent stage. Empirical support for this idea has been mixed. Implicit in both theory and empirical work is that the risk of mortality in the subsequent stage is unknown. However, some embryos and larvae of both vertebrates and invertebrates assess cues of post-transition predation risk and alter the timing of hatching or metamorphosis accordingly. Furthermore, although life history switch points of prey have traditionally been treated as discrete shifts in morphology or habitat, for many organisms they are continuous transitional periods within which the timing of specific developmental and behavioral events can be plastic. We studied red-eyed treefrogs (*Agalychnis callidryas*), which detect predators of both larvae and metamorphs, to test if plastic changes during the process of metamorphosis could reconcile the mismatch between life history theory and empirical data and if plasticity in an earlier stage transition (hatching) would affect plasticity at a subsequent stage transition (metamorphosis). We reared tadpoles from hatching until metamorphosis in a full-factorial cross of two hatching ages (early- vs. late-hatched) and the presence or absence of free-roaming predators of larvae (giant water bugs) and metamorphs (fishing spiders). Hatching age affected the times from oviposition to tail resorption and from hatching to emergence onto land, but did not alter responses to predators or developmental stage at emergence. Tadpoles did not alter their age at emergence or tail resorption in response to larval or metamorph predators, despite the fact that predators reduced tadpole density by ~30%. However, developmental stage at emergence and time needed to complete metamorphosis in the terrestrial environment were plastic and consistent with predictions of the "minimize μ/g" framework. Our results demonstrate that likely adaptive changes in life history transitions occur at previously unappreciated timescales. Consideration of plasticity in the developmental timing of ecologically important events within metamorphosis, rather than treating it as a discrete switch point, may help to reconcile inconsistencies between empirical studies of predator effects and expectations of long-standing ecological theory.

Key words: Agalychnis callidryas; *Anura; growth; life history switch point; mesocosm experiment; mortality; Neotropical treefrog; phenotypic plasticity; predator-induced variation; Smithsonian Tropical Research Institute, Gamboa, Panama; timing of metamorphosis.*

Introduction

As many as 80% of animals have complex life cycles (Werner 1988), wherein different life stages live in very different physical environments (e.g., aquatic larvae vs. terrestrial adults) or different habitats within a single environment (e.g., planktonic larvae vs. benthic adults). Researchers have long been interested in the ecological conditions that maintain such life cycles and determine the timing of transitions between stages. Wilbur and Collins (1973) noted plasticity in the timing of and size at metamorphosis in amphibians and proposed a model to describe when larvae should metamorphose, which was modified by Werner and Gilliam (1984) and Werner (1986). This theory predicts that organisms should maximize their chance of reaching a future size by minimizing the ratio of the mortality rate (μ) to growth rate (g) across stages; i.e., they should switch stages when μ/g in the next stage drops below that in the current stage (Werner and Gilliam 1984, Werner 1986). This can also be applied to other life history switch

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points such as timing of hatching (Sih and Moore 1993, Warkentin 1995).

Research spanning more than three decades and conducted across multiple systems has consistently demonstrated plasticity in the timing of hatching and metamorphosis (Benard 2004, Relyea 2007, Warkentin 2011*a*, *b*). The timing of these transitions can change as functions of the competitive and predatory environment as well as in response to abiotic conditions. Although classic life history theory predicts that larvae should metamorphose earlier in the presence of predators (Werner and Gilliam 1984, Werner 1986), many empirical tests have found that they actually do so later, or that predators have no effect on metamorphic timing (reviewed in Benard 2004, Relyea 2007). Fewer studies have examined variation in timing of hatching, but these studies demonstrate that many organisms can accelerate hatching in response to egg-stage risks and some delay hatching in response to larval-stage risks (reviewed in Warkentin 2011*a*, *b*).

One explanation for the incongruence of theory and data is that early studies did not appreciate the costs of predator-induced defenses that are now well documented (Benard 2006). In addition, studies have not appreciated that risk assessment and adaptive changes in the timing of life history transitions may occur over smaller time horizons. In fact, most ecological studies of amphibians treat the process of metamorphosis as a single discrete event (~75% of published studies; Walsh 2010) and have therefore focused on quantifying time to metamorphosis as the primary metric for developmental plasticity. However, this approach often lumps larval and metamorphic periods together and ignores adaptive variation that may occur during the transition itself (Downie et al. 2004). As in amphibians, life stage transitions in holometabolous and hemimetabolous insects are flexible processes, but often have been treated as single discrete events in ecological studies of life history plasticity (Hechtel and Juliano 1997, Peckarsky et al. 2002). Collectively, the flexible timing of developmental milestones and behaviorally mediated ecological switches during metamorphosis have been largely ignored, but such variation in developmental events may sometimes reflect adaptive responses to environmental conditions. Indeed, the few studies that have looked at variation in the duration of metamorphosis have found that it can be affected by predators (Van Buskirk and Saxer 2001, Walsh et al. 2008), as well as temperature (Pandian and Marian 1985) and phylogeny (Buchholz and Hayes 2002).

Another source of the discrepancy between theory and data may stem from differences in the time horizons over which information is accessible and decisions can be made. Most theoretical studies predict life history switch points assuming that decisions are optimized for evolutionarily established expectations of average survival and growth across stages (Wilbur and Collins 1973, Werner and Gilliam 1984, Werner 1988, Abrams and Rowe 1996, Day and Rowe 2002). However, in empirical studies, life history decisions may be induced by environmental cues that indicate both current and future growth and mortality risk over short time horizons (Van Buskirk and Saxer 2001, Vonesh and Warkentin 2006, Walsh et al. 2008, Touchon et al. 2013*b*). Moreover, if the growth and risk trade-off surfaces in each stage are nonlinear (Peacor et al. 2013), then Jensen's Inequality (Ruel and Ayres 1999) indicates that expectations based on average conditions experienced in each life history stage will not match observed patterns. This seems likely, given what we know about the functional response surfaces for post-hatching risk (McCoy et al. 2011) and about how risk changes around metamorphosis (Touchon et al. 2013*a*). Even in the absence of real-time information about post-transition risk, the way that animals estimate risk in the next stage may be dependent upon condition (e.g., larger animals with greater reserves may estimate risk differently than animals in poorer condition).

Touchon et al. (2013*a*) distinguish three forms of plasticity in metamorphosis, which apply to essentially all animals with complex life cycles. First, growth rate and development rate during the larval period may vary, affecting the timing of and size at initiation and completion of metamorphosis. These effects may be adaptive or simply a direct consequence of environmental variation, and they represent the type of variation described by Wilbur and Collins (1973). Second, development rate during the process of metamorphosis can vary, affecting the duration of the transition between larval and juvenile stages: for example, insect development rate can vary in response to multiple environmental factors such as photoperiod, temperature, predation risk, and food availability (Nylin and Gotthard 1998). Third, the timing of, and phenotype at, the movement between habitats can vary, e.g., crawling from water onto land in frogs (Touchon et al. 2013*a*) or settling from plankton to benthos. Ecologists should, therefore, think of metamorphosis as a stage or period, not a discrete transition point (Fig. 1). Like the larval period, both the duration of metamorphosis and the developmental timing of the habitat switch within it can be considered in the context of life history optimization theory and the "minimize μ/g" framework (sensu Werner and Gilliam 1984).

We use an anuran as a model to better understand developmental plasticity in metamorphosis. Anuran metamorphosis is well studied developmentally, as a coordinated combination of multiple processes (Shi 2000), and metamorphic climax is divided into multiple stages (i.e., Gosner stages 42–45; Gosner 1960). Nonetheless, little ecological research has examined sources of variation in the timing and duration of stages during the metamorphic process (Downie et al. 2004, Walsh 2010). Metamorphic climax begins with the emergence of forelimbs from the body (Gosner stage 42). Once forelimbs are external, the tail begins to

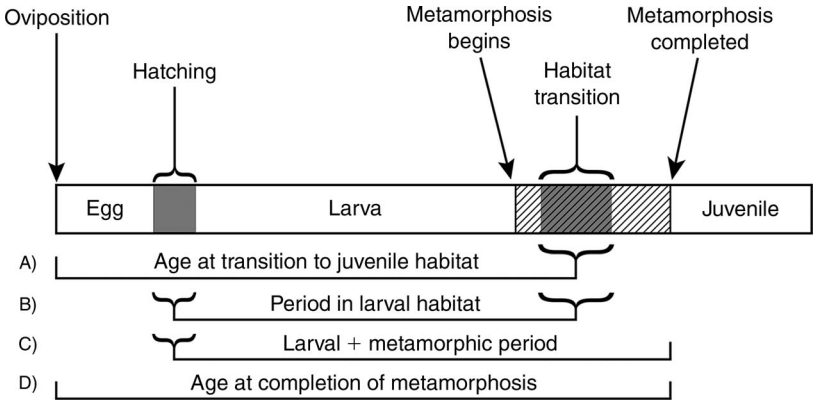

FIG. 1. Individual development from egg to juvenile is highly flexible in organisms with complex life cycles. Gray regions indicate discrete events with flexible timing; diagonal lines indicate metamorphosis. Hatching in many animals can occur at different points in time and development as a result of various factors (e.g., predators, pathogens). In addition to plasticity in the onset and completion of metamorphosis (not shown), animals can behaviorally alter the chronological and developmental timing (age and stage) of the transition from larval to juvenile environments that occurs during this period. Metamorphosis is not a single discrete event or time point, but rather a period of rapid development that is often partitioned between two environments. We tested effects of hatching age and predation risk on four partially overlapping periods, demarcated by developmental and behavioral events, that assay different elements of metamorphic plasticity: (A) from oviposition to the habitat transition (in amphibians, when metamorphs crawl from water onto land); (B) from hatching to the habitat transition; (C) from hatching to the completion of metamorphosis (in amphibians, when the tail is fully resorbed); and (D) from oviposition to the completion of metamorphosis.

atrophy, mouthparts transition from larval to juvenile form, and the animal is capable of leaving the aquatic habitat. Metamorphic climax is complete when the tail is fully resorbed (Gosner stage 46). We focus on responses of the red-eyed treefrog (*Agalychnis callidryas*) to variation in risk to determine whether a finer-scale examination of events during metamorphosis can provide insights to help reconcile the apparent mismatch between theory and past empirical work. *Agalychnis callidryas* tadpoles assess information about current and post-metamorphic predation risk, and can alter both the timing of metamorphosis and developmental stage at emergence. Vonesh and Warkentin (2006) found that caged fishing spiders (a semiterrestrial predator of froglets as they emerge from ponds) induced *A. callidryas* to delay the transition from water to land and to emerge at a larger size, compared to treatments with only a caged aquatic predator (water bug), or predator-free controls. They also found that animals left the water developmentally earlier in the metamorphic process, with longer tails, in response to water bug cues.

In the current study, we have three primary goals. First, we assess multiple components of metamorphic timing and phenotype for *A. callidryas* tadpoles raised with or without larval and/or metamorph predators. We used free-roaming predators in large mesocosms in order to evaluate the predictions of the "minimize μ/g" framework under seminatural conditions, which include changes in the density of prey as a result of predation. We provided high resource levels to reduce variation in g, thereby allowing us to focus on effects of variation in μ. Second, we tested whether metamorph traits and their responses to predators of larvae and metamorphs are altered by plastic decisions in an earlier life stage (by inducing premature hatching during the egg stage; Warkentin 1995, 2000). Lastly, because metamorphic timing has been assayed with various measures, (but most often as a single time point; Walsh 2010), we explicitly test whether different single-point measures provide different perspectives on potential changes in metamorphosis (Fig. 1). We predict that *A. callidryas* will leave the water earlier during metamorphic climax in response to both larval and metamorph predators, because doing so reduces predation risk during this dangerous period. Aquatic predation by water bugs increases once tadpoles' arms emerge. Spiders cue in on movement at the water surface and on land, and long-tailed metamorphs reduce their activity substantially upon emergence, making it advantageous to complete metamorphosis terrestrially (Vonesh and Warkentin 2006, Touchon et al. 2013*a*). We predict that hatching early would allow animals to reach metamorphic climax earlier and that these effects would be separate from effects of predators, because hatching prematurely has a growth and developmental benefit for *A. callidryas* (Willink et al. 2013).

MATERIALS AND METHODS

Experimental design

Red-eyed treefrogs are common in Neotropical wet forests from the Yucatan to Colombia (Duellman 2001). Adults lay eggs on plants over ponds and tadpoles drop into the water upon hatching. At our field site at the Smithsonian Tropical Research Institute in Gamboa, Panama, eggs generally hatch 6–7 days post-oviposition (dpo) if left undisturbed, but can hatch as early as 4 dpo

in response to egg-stage risks such as predators or fungal infection (Warkentin 1995, 2000, Warkentin et al. 2001). Hatching early increases embryo survival but often reduces larval survival, via decreased viability (Touchon et al. 2013*b*, Wojdak et al. 2014) and increased predation (Warkentin 1995, Willink et al. 2013).

Our experiment was conducted in 64 400-L plastic mesocosms (0.7 m diameter base, 0.9 m diameter mouth, 0.8 m high, with screened holes at 0.62 m height) in a partially shaded field at the forest edge. We manipulated three variables potentially important to larval *A. callidryas* survival and growth, and to timing of, and phenotype at, metamorphosis: hatching age, aquatic predation risk during the larval period, and terrestrial predation risk upon emergence from the water. Embryos were stimulated to hatch at either 4 days or 6 days post-oviposition (early- or late-hatched, respectively). We manipulated the presence of an aquatic predator (adult giant water bugs, *Belostoma* cf. *porteri*; Belostomatidae) and a semiterrestrial predator of froglets (fishing spiders, *Thaumasia* sp.; Pisauridae). Predators were free-roaming. Caged predator studies, while valuable, can inadvertently subsidize periphyton and phytoplankton communities, thereby muddying the effects of predators even in experiments designed to isolate their effects (Costa and Vonesh 2013). Water bugs were replaced if they died, and spiders were replaced if they had egg sacs or died. We initially replaced spiders that were killed or seriously injured by water bugs, but due to the high level of intraguild predation (IGP) and constraints on spider availability, we stopped replacing spiders in tanks with water bugs after 11 days (0–6 spiders replaced per tank, 3.3 ± 0.4 spiders, mean ± SE; seven replacement dates at 1–3 day intervals, 1.6 ± 0.3 d, mean ± SE; last remaining spiders removed after 14 days). In tanks without water bugs, spiders were present until the end of the experiment. The experiment lasted until all tadpoles died or metamorphosed.

We conducted a full-factorial cross of the three variables for eight treatment combinations (two hatching ages × two larval predator treatments levels × two metamorph predator treatments) set up in eight fully replicated spatial blocks ($N = 64$ experimental units). Three tanks were not set up properly (two from the late hatched–larval predator–no metamorph predator treatment and one from the late hatched–larval predator–metamorph predator treatment), and so were excluded from the experiment for a final $N = 61$ mesocosms. We filled mesocosms 3–5 days before the experiment began with a mixture of captured rainwater and aged tap water and fitted them with screen covers to prevent colonization by unwanted organisms. To promote an aquatic community in each mesocosm, we added 250 g of leaf litter collected from nearby Experimental Pond (9°7′14.88″ N, 79°42′14.11″ W) and a 1-L inoculate of zooplankton and phytoplankton collected from Ocelot Pond (9°6′8.62″ N, 79°40′56.96″ W). To make it easier to find tadpoles during censuses, 80% (200 g) of the leaf litter was contained in a mesh bag. Due to variation in tree canopy above mesocosms, different blocks experienced different amounts of shading, but replicates within each block experienced similar shading.

We collected 98 freshly laid *A. callidryas* clutches (~40 eggs each) from Experimental Pond (blocks 1–5) and Ocelot Pond (blocks 6–8) on the morning of 11 July 2010. All individuals in the experiment came from eggs laid on the same night. We maintained clutches in an open-air laboratory and misted them regularly with aged tap water to maintain hydration, and randomly assigned half to each hatching treatment. When embryos were 4 or 6 days post-oviposition, as appropriate, at ca. 11:00 hours, clutches were submerged in a single water container for each source pond, and eggs were gently rubbed with a finger to induce hatching, allowing tadpoles to mix in the water. Early-hatched tadpoles were added to mesocosms on 15 July and late-hatched tadpoles were added on 17 July.

We haphazardly drew groups of 50 hatchlings from the pooled tadpoles from either pond, digitally photographed them in a shallow tray with a ruler, and added them to each mesocosm. We added 2.5 g of Sera micron powder (Sera, Heinsberg, Germany) to each mesocosm every five days as a resource supplement for tadpoles. This resource level was higher than that used in our previous studies in this system (McCoy et al. 2011, Touchon et al. 2013*b*), and was chosen to minimize variation in growth rates (g), even after thinning by predators, more clearly allowing us to examine effects of mortality risk (μ). To monitor tadpole growth and survival, we dip-netted all tadpoles out of each mesocosm on 23 July and 2 August (i.e., 12 and 22 dpo) and photographed them. Tadpoles were censused by dip-netting until 10 successive dips yielded no additional tadpoles. Tadpole total length (snout to tail tip) at hatching and at each census was measured from photographs using ImageJ digital image analysis software (Rasband 2012).

Metamorph predators (fishing spiders) were added to tanks on 2 August (22 dpo) along with a layer of floating vegetation, which served as a substrate for spiders. We subsequently visually assessed daily the limb development in tanks with the most advanced tadpoles. Forelimb emergence began on 13 August and emergence from the water began on 14 August, after which we thoroughly checked all tanks for emerged metamorphs each morning. Metamorphs typically slept on the inner lip of the tank or on plants. We focused on timing of emergence from the water and did not attempt to collect data on forelimb emergence; the latter would have required dipping mesocosms daily, which was unfeasible and such handling stress at Gosner stage 41 or 42 could potentially alter the timing of forelimb emergence or emergence from the water. After collection, metamorphs were brought to the open-air laboratory and were housed individually in 266-mL cups (~7 cm diameter, 7 cm high) with perforated lids to complete tail

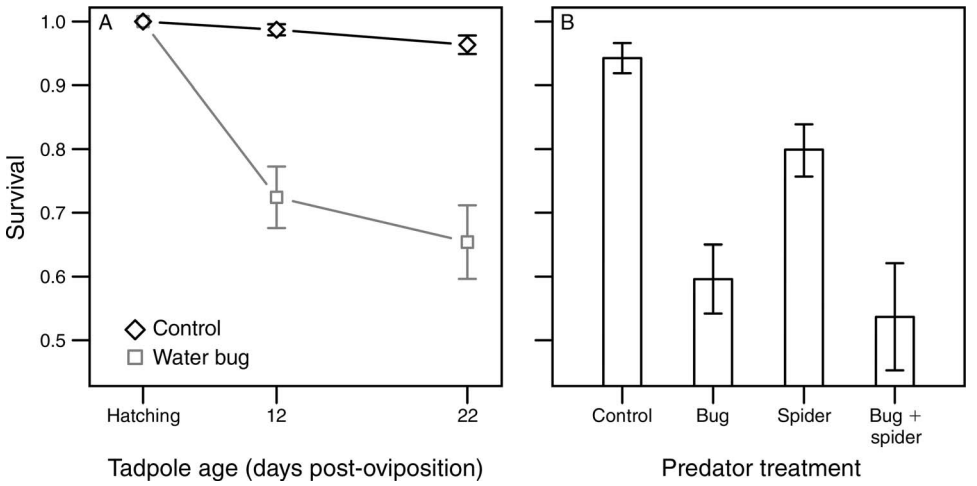

FIG. 2. Survival of red-eyed treefrogs *Agalychnis callidryas*, (A) as larvae and (B) through the morning after metamorphs emerged from the water, was affected by larval and metamorph predators (giant water bugs and fishing spiders, introduced alone and together). Larval survival was measured at 12 and 22 days post-oviposition. Because hatching age did not significantly affect survival, early- and late-hatching treatments are pooled. Data shown are means (with 95% confidence intervals) for tanks, controlling for random effects of variation between blocks and tanks within blocks.

resorption (Gosner stage 46, no visible tail stub remaining). Cups contained a few milliliters of aged tap water to maintain metamorph hydration. We measured tail length, snout–vent length (SVL), and Gosner stage as early as possible on the day of emergence. Based on our extensive observations, we also defined a stage 43.5, based on mouth development (corner more than halfway from nare to eye). We checked every metamorph daily, remeasured them when tail resorption was complete, and calculated the time from emergence through tail resorption. We released all froglets at their pond of origin.

Statistical analyses

All analyses were conducted in R v3.0.2 (R Development Core Team 2013). All analyses were linear or generalized linear mixed models (LMM and GLMM, respectively) using the lme4 package (Bates et al. 2013). For all response variables, we used Akaike's information criterion (AIC) to choose the best model, and then estimated P values of factors and their interactions using likelihood ratio tests. Each set of models always contained the three factorial predictors and their interactions, as well as other appropriate predictors (e.g., age or size at emergence from the water for post-metamorphic traits, number of tadpoles alive at the second census for effects of thinning). Analyses of tail length at emergence included SVL at emergence as a covariate, but not age at emergence. SVL and age at emergence are correlated and both may affect tail length, but SVL has a more direct effect on metamorph tail length. Tadpole total length, age, tail length and SVL at emergence, and time from emergence until completion of tail resorption were all log-transformed for analyses. All analyses except survival used data from individuals, with "Block" and "Tank within Block" as random effects to control for nonindependence of individuals within tanks. Because values for survival were tank values (proportion of animals surviving out of the initial 50), the only random effect was Block. We initially included pond of origin as a random effect, but the effect size was always effectively zero; thus we removed pond from the final analyses. Age of tadpoles or metamorphs was measured as number of days post-oviposition. However, we also conducted analyses using aquatic period (from hatching until emergence), because early-hatched tadpoles entered mesocosms two days prior to late-hatched tadpoles. As a complement to our mixed-model approach, we also analyzed data on Gosner stage at emergence using a chi-square goodness-of-fit test, testing the proportions of individuals emerging at different developmental stages (see Appendix). For the sake of brevity, we only report results of the primary predictors from each model and significant interactions, but not nonsignificant interactions. See the Appendix for the full structure of all models.

RESULTS

Survival

Tadpole survival was significantly affected by the presence of water bugs, and that effect changed over time, but survival was not affected by hatching age (Fig. 2A; larval predator, $\chi^2 = 86.3$, $P < 0.00001$; time, $\chi^2 = 15.7$, $P = 0.00007$; larval predator × time, $\chi^2 = 8.5$, $P = 0.004$; hatching age, $\chi^2 = 0.47$, $P = 0.49$). Water bugs reduced survival by approximately 25% by the first census, and slightly more than 30% by the second census (Fig. 2A).

Of 3050 tadpoles that began the experiment, 2172 survived to be collected as metamorphs (71.2% survival).

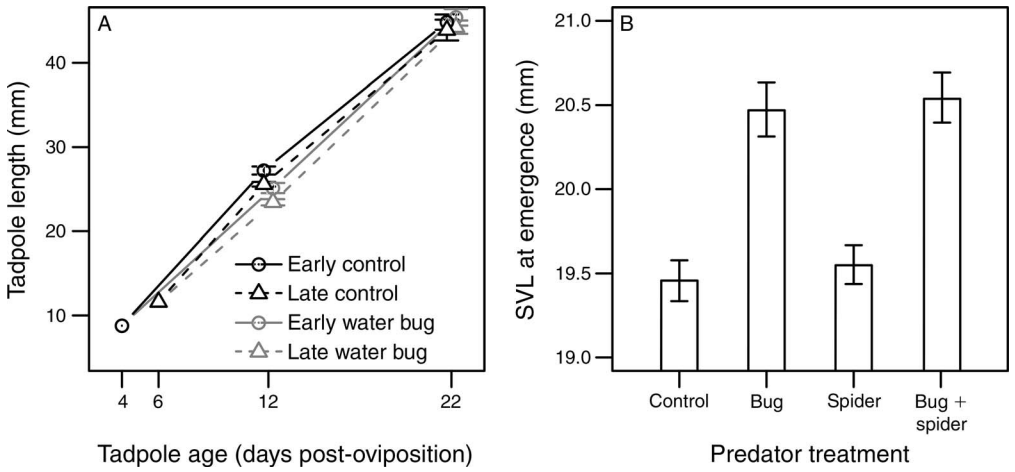

FIG. 3. Effects on size of *Agalychnis callidryas* tadpoles during (A) the larval period and (B) at emergence from the water during metamorphosis. Larval size was affected by both the presence of larval predators and hatching age (number of days post-oviposition). Size at emergence (SVL, snout–vent length) was affected by larval predators but not by metamorph predators. Data shown are means (with 95% confidence intervals) for tanks, controlling for random effects of blocks and tanks within blocks.

Survival until the morning after emergence was significantly reduced by the presence of both larval and metamorph predators, and their effects were not independent (Fig. 2B; larval predator, $\chi^2 = 64.8$, $P < 0.00001$; metamorph predator, $\chi^2 = 19.3$, $P = 0.00001$; larval predator × metamorph predator, $\chi^2 = 14.1$, $P = 0.0002$). Spiders reduced tadpole survival by ~15% in the absence of water bugs, but only by 6% in their presence. Hatching age did not affect survival to emergence ($\chi^2 = 0.8$, $P = 0.36$). When examining the number of *A. callidryas* that died after the second census, we found the highest mortality in the spider-only treatment (Appendix: Fig. A1; larval predator, $\chi^2 = 0.4$, $P = 0.54$; metamorph predator, $\chi^2 = 27.7$, $P < 0.00001$; larval predator × metamorph predator, $\chi^2 = 16.3$, $P = 0.00005$). Predation did not differ if water bugs were alone or with spiders.

Growth and size

Tadpole size was strongly influenced by hatching age, larval predator treatment, time, and the interactions of time with larval predators and of time with hatching age, indicating that the effects of hatching timing and predator presence on size changed over the course of the experiment (Fig. 3A; hatching age, $\chi^2 = 37.5$, $P < 0.00001$; larval predator, $\chi^2 = 20.3$, $P = 0.00001$; time, $\chi^2 = 46.0$, $P < 0.00001$; hatching age × time, $\chi^2 = 10.4$, $P = 0.0007$; larval predator × time, $\chi^2 = 38.9$, $P < 0.00001$). The interactions were driven by the fact that, at 12 dpo, tadpoles with predators were significantly smaller than those in control tanks, and early-hatched tadpoles were significantly larger than late-hatched tadpoles, but by 22 dpo, all tadpoles were similarly sized (Fig. 3A). We did not test for an effect of metamorph predators because they were added to tanks after the final larval census.

The SVL of *A. callidryas* metamorphs when leaving the water was influenced by age at emergence onto land (dpo) and larval predators, but not by hatching age or metamorph predators (Fig. 3B; age at emergence, $\chi^2 = 130.6$, $P < 0.00001$; larval predator, $\chi^2 = 29.9$, $P < 0.00001$; metamorph predator, $\chi^2 = 0.5$, $P = 0.50$; hatching age, $\chi^2 = 0.7$, $P = 0.42$). Specifically, animals leaving the water later were larger, and metamorphs reared as tadpoles with water bugs were larger than controls (Fig. 3B). The effect of water bugs probably was due to thinning. The number of tadpoles alive in a tank at the second census was strongly related to the mean SVL at tail resorption of metamorphs, but neither water bugs nor spiders had effects separate from tadpole density (Appendix: Fig A2; tadpole density, $\chi^2 = 46.5$, $P < 0.00001$; larval predator, $\chi^2 = 1.4$, $P = 0.2$; metamorph predator, $\chi^2 = 0.2$, $P = 0.7$).

Plasticity in timing of emergence, tail resorption, and duration of metamorphosis

The first metamorphs emerged from the water at 34 dpo and the last at 64 dpo (40 ± 3.2 dpo, mean ± SD). In addition to emergence timing, we considered the length of the developmental period through completion of tail resorption, starting both from oviposition (embryonic + larval + metamorphic periods) and from hatching (larval + metamorphic periods; Fig. 1).

The timing of metamorph emergence, in terms of age measured from oviposition, did not vary in response to larval or metamorph predators, but was influenced by hatching age; animals from eggs that had hatched early emerged 1.2 days earlier than those that had hatched late (Fig. 4A; hatching age, $\chi^2 = 21.8$, $P < 0.00001$; larval predator, $\chi^2 = 0.09$, $P = 0.77$; metamorph predator, $\chi^2 = 0.4$, $P = 0.55$). Conversely, because early-hatched tadpoles entered mesocosms two days before late-hatched tadpoles, they actually spent longer in the water; the time from hatching to emergence was, on average, approximately 1 day longer for early-hatched

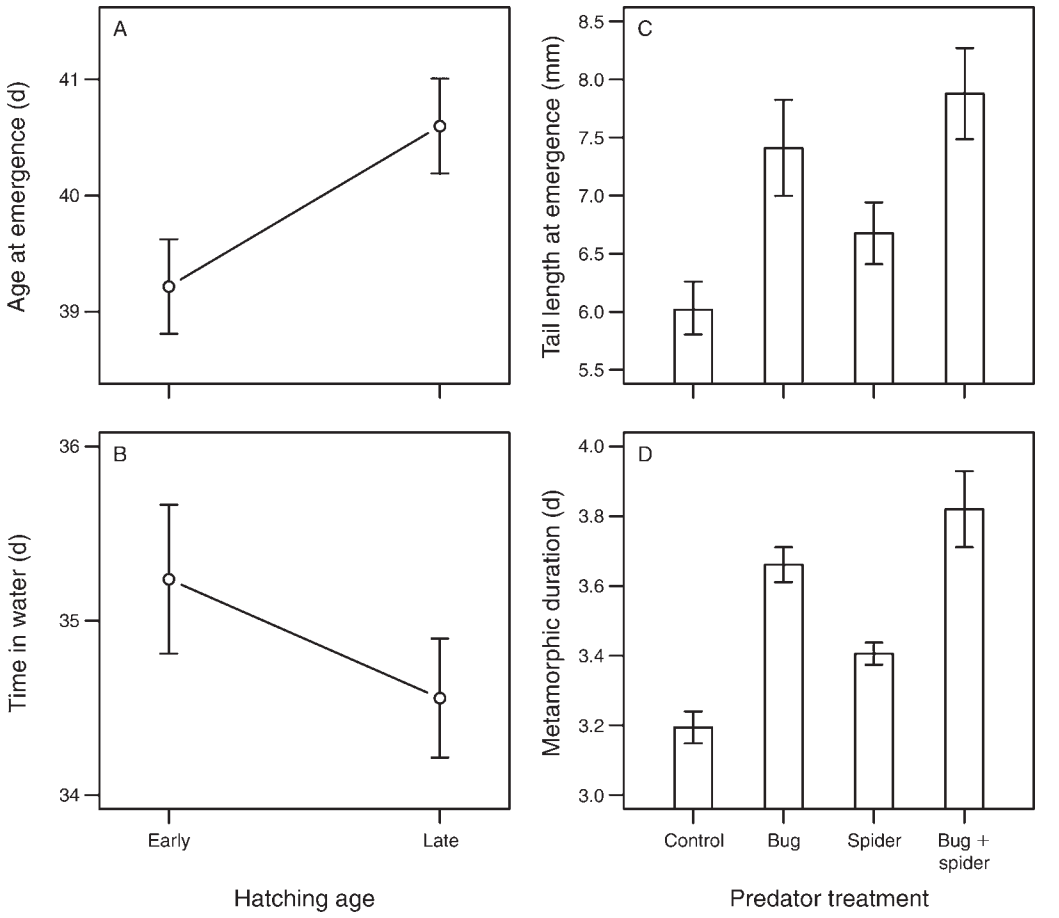

FIG. 4. (A) The age of *Agalychnis callidryas* at emergence onto land, in number of days post-oviposition, was affected by hatching age. (B) Similarly, the aquatic period (from hatching to emergence) was also affected by hatching age, but the pattern was the reverse. (C) Larval and metamorph predators interacted to affect tail length at emergence from water, after controlling for effect of size. (D) Larval and metamorph predators also independently affected duration of the post-emergence portion of metamorphosis, after controlling for variation in tail length at emergence. Data shown are means (with 95% confidence intervals) for tanks, controlling for random effects of variation between blocks and tanks within blocks.

than for late-hatched individuals (Fig. 4B; $\chi^2 = 15.3$, $P = 0.00009$). Thus, although early-hatched tadpoles enter the larval environment earlier, the developmental benefit of this is only partially realized.

Hatching age was also the primary predictor of the time from oviposition through tail resorption, and the effect of larval predators was marginally nonsignificant (hatching age, $\chi^2 = 19.9$, $P < 0.00001$; larval predator, $\chi^2 = 3.3$, $P = 0.07$). Metamorph predator treatment had no effect ($\chi^2 = 0.2$, $P = 0.66$). The time from hatching through tail resorption was also affected by hatching age, but not by larval or metamorph predators (hatching age, $\chi^2 = 17.6$, $P = 0.00003$; larval predator, $\chi^2 = 2.3$, $P = 0.13$; metamorph predator, $\chi^2 = 0.3$, $P = 0.59$). Both effects were in the same direction as for timing of emergence; early-hatched animals finished metamorphosis younger in terms of time since oviposition, but later in terms of time since hatching.

Controlling for the direct effect of size, the tail length of newly emerged metamorphs indicates when animals choose to leave the water during their transition from tadpole to froglet. Tail length increased with SVL, varied as a result of both larval and metamorph predator presence, and was not affected by hatching age (Fig. 4C; SVL, $\chi^2 = 72.8$, $P < 0.00001$; larval predator, $\chi^2 = 44.9$, $P < 0.00001$; metamorph predator, $\chi^2 = 16.2$, $P = 0.00006$; hatching age, $\chi^2 = 1.4$, $P = 0.24$). In addition, the interaction between larval and metamorph predators was significant, indicating that the effect of spiders on metamorph tail length was greater in the absence of water bugs ($\chi^2 = 5.6$, $P = 0.018$). Averaging across other treatments, tadpoles in the presence of water bugs left the water with 19% longer tails than controls. Similarly, tadpoles exposed to spiders emerged with 8% longer tails than those not so exposed, and the spider effect was stronger in the absence of water bugs, when spiders were present throughout the full period of metamorph emergence (10% vs. 4% longer; Fig. 4C).

Similar to the finer-grained data on tail length at emergence, Gosner stage at emergence varied with larval and metamorph predators (Appendix: Fig A3). Nearly all *A. callidryas* left the water between Gosner stages 43 and 44. Individuals raised with any predators were more likely to emerge developmentally earlier, at stage 43, whereas tadpoles raised without predators were more likely to emerge later, at stages 43.5 or 44 (Appendix; χ^2 goodness-of-fit test, $\chi^2 = 41.2$, $P < 0.00005$). There was no effect of hatching age on stage at emergence ($\chi^2 = 5.8$, $P = 0.22$).

The duration of the terrestrial portion of metamorphosis (the days needed to fully resorb the tail after emerging from the water) was strongly linked to tail length at emergence, but also was independently influenced by larval and metamorph predators (Fig. 4D; tail length, $\chi^2 = 222.8$, $P < 0.00001$; larval predator, $\chi^2 = 33.4$, $P < 0.00001$; metamorph predator, $\chi^2 = 10.1$, $P = 0.001$). After controlling for differences in tail length at emergence, metamorphs raised with larval predators took 12% longer to complete metamorphosis than those raised as controls, and metamorphs that emerged with spiders took 5% longer than metamorphs without spiders (Fig. 4D). There was also a marginally nonsignificant effect of hatching age, with late-hatched animals taking slightly less time to complete metamorphosis (hatching age, $\chi^2 = 3.3$, $P = 0.07$).

Discussion

Theory predicts that organisms should time transitions from the larval to adult environments to minimize the ratio of mortality risk to growth rate (Wilbur and Collins 1973, Werner and Gilliam 1984, Werner 1986). Thus, larvae at risk of predation should transition at a smaller size than larvae growing in the absence of predators. Despite the fact that for many animals, predation risk is high during the period of metamorphosis, e.g., amphibians (Arnold and Wassersug 1978), insects (Corbet 1962), marine invertebrates (Gosselin and Qian 1997), and reef fishes (Almany and Webster 2006), much empirical data remains at odds with this prediction.

We tested the qualitative predictions of the "minimize μ/*g*" framework for metamorphosis with an organism that can simultaneously detect the presence of both larval and metamorph predators. Our study organism, *A. callidryas*, did not change the timing of metamorphosis as a singular event (either time to emergence from the water or time to tail resorption) in response to predators. It did, however, flexibly alter multiple aspects of the process of metamorphosis. Metamorphs left the aquatic environment developmentally earlier during the period of metamorphosis in the presence of both larval and metamorph predators, although the effect of water bugs was decidedly stronger than that of spiders (Fig. 4C). Based on our previous studies with *A. callidryas* (Touchon et al. 2013*a*), these responses are what would be expected in order to minimize μ/*g*. For *A. callidryas* tadpoles, the risk of predation by water bugs is high just after hatching (Willink et al. 2013), decreases substantially with growth (McCoy et al. 2011), and then increases dramatically with eruption of forelimbs from the body (Touchon et al. 2013*a*). In addition, Touchon et al. (2013*a*) found that immediate risk from spiders after leaving the water was lowest for metamorphs with longer tails, due to their lower activity. Thus, in transitioning from aquatic to terrestrial environments, *A. callidryas* adjusts the timing of events in a manner consistent with reducing predation risk in both habitats. Furthermore, exposure to both larval and metamorph predators extended the duration of the metamorphic period after emergence onto land, even after controlling for predator-induced variation in tail length at emergence, suggesting a physiological change affecting development rate (Fig. 4D).

Although we cannot separate the effects of water bugs and spiders when they co-occurred, risk from spiders alone exceeded the risk when spiders were paired with water bugs (Fig. 2B; Appendix: Fig. A1). This risk reduction effect of water bugs on spiders probably occurred primarily through direct predation, although intimidation also may have been a factor. The stronger effect of water bugs than of spiders on the developmental timing of emergence does not match the relative magnitude of their effects on mortality after our second census, but it is consistent with the much greater risk from bugs than spiders in individual metamorph predation trials (Touchon et al. 2013*a*). Because both effects were in the same direction (earlier emergence; Fig. 4C), given the strong response to water bugs alone, there may have been no advantage to increasing the response magnitude when spiders were also present. However, risk reduction due to intra-guild predation could also have contributed to the less-than-additive response with both predators.

Agalychnis callidryas individuals took longer to complete the terrestrial portion of metamorphosis (from emergence onto land until their tail was fully resorbed) if they had been exposed to larval or metamorph predators. Although flexibility in larval period is well known across taxa, e.g., dipterans (Hechtel and Juliano 1997), odonates (Johansson et al. 2001), reef fishes (Bay et al. 2006), amphibians (Gomez-Mestre and Buchholz 2006), and sponges (Whalan et al. 2008), few studies have examined the duration of metamorphosis as a plastic trait with the potential for functionally relevant variation. Only two studies that we know of have explicitly assessed larval predator-induced variation in the duration of the metamorphic process, finding accelerated metamorphosis in the presence of a predator, at a cost of greater mass loss during the process (Van Buskirk and Saxer 2001, Walsh et al. 2008). It is important to note that the terrestrial portion of metamorphosis that we measured took place in a predator-free environment (plastic cups in the lab); thus, predator effects are carried over from prior

exposure, and are not responses to current cues. For the strongest effect that we document here, that of water bugs, this would necessarily be the case after metamorphs leave the aquatic environment. Spider effects might have been stronger, or different, with continued cues, although terrestrial *A. callidryas* metamorphs do not change their activity level in the presence of spiders (Touchon et al. 2013*a*) and, in nature, at least some individuals probably also move out of fishing spider habitat before completing tail resorption.

The variation that we found in the duration of metamorphosis may have adaptive or nonadaptive explanations. For instance, metamorphs might make adaptive heterokairic shifts in the metamorphic process, i.e., change the relative timing of different developmental events (Spicer and Burggren 2003). Alternatively, the stress of prior exposure to predators may affect metabolic processes or shift resources away from metamorphosis itself, thereby having nonadaptive consequences on the rate of tail resorption. Further detailed research will be needed to elucidate the specific mechanisms that underlie the variation that we document here.

Water bugs were effective predators of *A. callidryas* tadpoles (Fig. 2B), but the resulting reduction in larval density only affected size at metamorphosis and not time to metamorphosis. Thus, our data indicated that larval predators affected growth but not development (Gomez-Mestre et al. 2010). When predators are allowed to reduce prey density, resources become more abundant for the surviving prey, often resulting in increased growth rates (Van Buskirk and Yurewicz 1998, Relyea 2002). Thinning also can indirectly increase prey resources via enhanced periphyton growth (Costa and Vonesh 2013). Resources were intentionally abundant in all of our tanks (67% greater than the "high" resource level defined in Touchon et al. [2013*b*]), and tadpoles in this experiment may have been near the upper limit of larval development rate, regardless of tadpole density, such that additional resources caused no further increase. In support of this, metamorphs emerged, on average, 40 days after oviposition across all treatments, substantially faster than in any previous study of this species; average time to metamorphosis has been given as 69 days (Warkentin 1999), 55–58 days (Vonesh and Warkentin 2006), and 49–59 days (Touchon et al. 2013*b*).

Interestingly, we found no effects of predators on timing of emergence from the water, unlike the study of Vonesh and Warkentin (2006), in which *A. callidryas* tadpoles delayed metamorphosis to emerge at a larger size in response to the presence of caged spiders. The later addition of spiders in our experiment may have occurred after developmental timing was set (Hensley 1993, Leips and Travis 1994), but Touchon et al. (2013*a*) found no effect of metamorph size on predation by spiders or water bugs. The increased size of metamorphs with free-roaming water bugs in our experiment, as opposed to the decrease with caged bugs in Vonesh and Warkentin (2006), presumably reflects our richer resource environment and effects of thinning. Moreover, in the study by Vonesh and Warkentin (2006), spiders and water bugs were caged, present equal amounts of time, and fed on similar schedules (thereby creating similar amounts of chemical cues in the water); in contrast, our spiders were present less than half the amount of time as water bugs and only fed opportunistically. Furthermore, our free-roaming water bugs could interact behaviorally with tadpoles in the water even when not directly preying upon them, whereas spiders stayed mostly above the water surface. The fact that we still see significant effects of spiders on metamorph phenotype therefore reflects their importance as a predator during the critical transition to land.

Unlike a previous study with *A. callidryas* (Touchon et al. 2013*b*), variation in age at hatching did not have detectable effects on metamorph size. Plasticity in hatching timing did affect larval growth such that early-hatched tadpoles were larger than late-hatched tadpoles at the first census (Fig. 3A). However, this effect had disappeared by our second census and, likewise, was undetectable at metamorphosis. Effects of hatching age on growth are relatively subtle and probably were outweighed by strong predation by water bugs and rich resources that contributed to tadpoles growing near their upper limits. Indeed, the nuanced developmental and ecological effects of variable hatching timing are likely to be more detectable when environmental conditions promote greater competition and thereby exacerbate early differences in growth and vulnerability (McCoy et al. 2011). It is interesting that the timing of key events (i.e., emergence, tail resorption) relative to oviposition and hatching was affected by hatching age, but not by predators, whereas phenotype at emergence and subsequent rate of development through metamorphosis were strongly affected by predators, but only marginally, if at all, by hatching age. This suggests that, at least under high resource conditions, the timing of entry into the larval environment might set, or set bounds on, aspects of development that play out over longer timeframes (Hensley 1993, Leips and Travis 1994). Nonetheless, within those constraints, animals can fine-tune their ecological transitions based on phenotype-dependent risk functions and environmental cues. Moreover, at least under some conditions, development rate during metamorphosis may be more sensitive to predators than is development rate through the larval period.

Conclusions

The "minimize μ/g" framework has been very valuable for helping ecologists to think about the evolution of complex life histories, but substantial empirical work stands at odds with theoretical predictions (reviewed in Benard 2004, Relyea 2007). We demonstrate that viewing metamorphosis as a process

with multiple components that may be optimized, instead of as a simple developmental marker that must be crossed, may help to reconcile these quixotic results. Had we only measured metamorphosis as a point in time, either when froglets crawled out of the water or when the tail was fully resorbed, our results would have been among the many that disagree with predictions from theory that organisms should adjust life history transitions in response to risk. However, by examining flexibility within the process of metamorphosis, specifically the developmental timing of emergence and the length of the terrestrial portion of metamorphosis, we show that red-eyed treefrog tadpoles alter their shift from water to land in a direction that reduces predation risk. Furthermore, by using a species that can assess information from post-metamorphosis predators, we demonstrate that this important life history transition can be further refined. It seems likely that many other organisms can perceive risk from post-metamorphosis threats and that this has simply been unappreciated and overlooked until now.

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Supplemental Material

Ecological Archives

The Appendix is available online: http://dx.doi.org/10.1890/14-1301.1.sm