Effects of plastic hatching timing carry over through metamorphosis in red-eyed treefrogs

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Abstract. Environmentally cued plasticity in hatching timing is widespread in animals. As with later life-history switch points, plasticity in hatching timing may have carryover effects that affect subsequent interactions with predators and competitors. Moreover, the strength of such effects of hatching plasticity may be context dependent. We used red-eyed treefrogs, *Agalychnis callidryas*, to test for lasting effects of hatching timing (four or six days post-oviposition) under factorial combinations of resource levels (high or low) and predation risk (none, caged, or lethal *Pantala flavescens* dragonfly naiads). Tadpoles were raised in 400-L mesocosms in Gamboa, Panama, from hatching until all animals had metamorphosed or died, allowing assessment of effects across a nearly six-month period of metamorphosis. Hatching early reduced survival to metamorphosis, increased larval growth, and had context-dependent effects on metamorph phenotypes. Early during the period of metamorph emergence, early-hatched animals were larger than late-hatched ones, but this effect attenuated over time. Early-hatched animals also left the water with relatively longer tails. Lethal predators dramatically reduced survival to metamorphosis, with most mortality occurring early in the larval period. Predator effects on the timing of metamorphosis and metamorph size and tail length depended upon resources. For example, lethal predators reduced larval periods, and this effect was stronger with low resources. Predators affected metamorph size early in the period of metamorphosis, whereas resource levels were a stronger determinant of phenotype for animals that metamorphosed later. Effects of hatching timing were detectable on top of strong effects of larval predators and resources, across two subsequent life stages, and some were as strong as or stronger than effects of resources. Plasticity in hatching timing is ecologically important and currently underappreciated. Effects on metamorph numbers and phenotypes may impact subsequent interactions with predators, competitors, and mates, with potentially cascading effects on recruitment and fitness.

Key words: Agalychnis callidryas; Anura; carry-over effects; Gamboa, Panama; latent effects; life-history switch point; Neotropical treefrog; Pantala flavescens; phenotypic plasticity.

INTRODUCTION

Most animals begin life as eggs. Thus, their first critical life-history switchpoint is hatching. While environmentally cued plasticity in later switch points, such as metamorphosis, has long been appreciated (e.g., Lynn and Edelman 1936), hatching has often been treated as a developmental event that occurs at a fixed stage, with any variation in hatching assumed to result from passive processes. Furthermore, the consequences of variation in hatching timing have remained largely unexamined. Recent syntheses highlight the number and diversity of cases of environmentally cued plasticity in

the timing and developmental stage of hatching (Christy 2011, Doody 2011, Martin et al. 2011, Oyarzun and Strathmann 2011, Warkentin 2011*a, b*, Whittington and Kearn 2011, Rafferty and Reina 2012). Hatching plasticity has been documented in response to many environmental factors including egg predators and pathogens, larval predators, physical risks to eggs or larvae, larval food resources, and conspecifics (Warkentin 2011*a, b*). Well-documented cases are spread phylogenetically throughout bilateria, including examples from trematodes, platyhelminthes, molluscs, polychaetes, nematodes, spiders, crustaceans, insects, echinoderms, fishes, amphibians, squamates, turtles, crocodilians, and birds (reviewed in Warkentin 2011*a*).

In amphibians, currently the best studied taxa with regards to hatching plasticity, there is no evidence for invariant hatching. All 38 amphibian species analyzed to date demonstrate some degree of hatching plasticity (Warkentin 2011*b*); however, the factors to which

species respond, the strength of responses, and the likely adaptive value of responses all vary. We do not yet know if hatching plasticity is typical or is a minority pattern across animals, but the accumulation of evidence indicates the need to assess the ecological consequences of hatching timing for later life stages.

Although studies of plasticity in hatching are still relatively few, studies of plasticity in metamorphosis spanning more than 70 years have revealed that there are carryover effects of the larval environment even after crossing this life-history switchpoint (Pechenik 2006). Many taxa respond to larval conditions by altering their metamorphic phenotype (e.g., size, morphology, larval period duration; Werner and Gilliam [1984], Benard [2004]), which can have important consequences for post-metamorphic growth (Van Allen et al. 2010), lifetime fitness (Semlitsch et al. 1988, McCoy et al. 2007), and population and community dynamics (Beckerman et al. 2002, McCoy et al. 2009). Such carryover effects have been demonstrated in bryozoans, gastropods, polychaetes, crustaceans, echinoderms, urochordates, insects, and amphibians (reviewed in Pechenik 2006). While ecologists now appreciate that plasticity in metamorphosis can link processes across life stages, our understanding of how effects of the embryonic environment carry over to affect performance in subsequent stages via hatching plasticity is in its infancy. This is a critical gap to address. Because hatching occurs early in life, carryover effects mediated by hatching plasticity may cascade through multiple life stages to alter later performance, phenotypes, and switchpoints (e.g., larval growth, morphology, metamorphosis, migration, onset of reproduction).

Studies that have examined carryover effects of hatching plasticity indicate that altered hatching timing can affect larval behavior (Warkentin 1999a), morphology (Warkentin 1999b), growth (Warkentin 1999b, Vonesh and Bolker 2005, Orizaola et al. 2010), metamorphic size (Vonesh and Bolker 2005, Touchon and Warkentin 2010), and morphology (Capellán and Nicieza 2007), as well as interactions with predators of larvae (Warkentin 1995, Vonesh and Osenberg 2003, Touchon and Warkentin 2010) and metamorphs (Vonesh 2005b). However, results from some of these studies conflict, suggesting that consequences of hatching timing may differ when assessed at different points in ontogeny. For example, most short-term studies with red-eyed treefrogs reveal immediate costs of early hatching via increased vulnerability to larval predators (Warkentin 1995, 1999a, but see McCoy et al. 2011). In contrast, in longer-term studies with spiny reed frogs (*Hyperolius spinigularis*), early-hatched tadpoles grew faster through vulnerable size classes and experienced less mortality from predators over the larval period as a whole (Vonesh and Osenberg 2003, Vonesh and Bolker 2005).

This body of work also indicates that the consequences of hatching plasticity may depend on environmental context. As with variation in egg size (Berven and Chadra 1988, Semlitsch and Gibbons 1990), effects of hatching timing can depend on the presence and identity of predators in the next life stage (Warkentin 1995, 1999a, Vonesh and Osenberg 2003, Vonesh and Bolker 2005, Touchon and Warkentin 2010, McCoy et al. 2011). Because hatching timing can vary with larval resources (e.g., Clare 1997, Whittington and Kearn 2011) and conspecific density (e.g., Livdahl et al. 1984, Kahan et al. 1988), we might expect its consequences to also depend on resource level or competition in the subsequent life stage. Furthermore, because effects of predators and resources on prey growth and survival are typically not independent (Wilbur 1988, Gurevitch et al. 2000), we might also expect interactions between hatching timing, predation, and resource availability.

The red-eyed treefrog, *Agalychnis callidryas*, is among the best-studied cases of hatching plasticity. Arboreally laid *A. callidryas* embryos hatch up to 30% early in response to attacks by egg-eating snakes and wasps (Warkentin 1995, 2000b), fungal infection (Warkentin et al. 2001), and flooding (Warkentin 2002). In *A. callidryas* and five related species, hatching timing involves a clear short-term trade-off; early hatchlings escape from threats to eggs but are more vulnerable to aquatic predators than are full-term hatchlings (Warkentin 1995, 1999a, Gomez-Mestre et al. 2008). Early hatchlings also begin feeding sooner and initially grow faster than their later-hatched siblings (Warkentin 1999b). *Agalychnis callidryas* larvae also alter growth rate and timing of metamorphosis in response to cues from predators (Vonesh and Warkentin 2006). Predation on *A. callidryas* larvae is greatest early in ontogeny (McCoy et al. 2011) and their growth rates vary with resource level (Gomez-Mestre et al. 2010) and density (S. S. Bouchard, C. R. Jenney, J. F. Charbonnier, and K. M. Warkentin, *unpublished data*), suggesting that long-term consequences of hatching timing may vary across larval environments.

Here we use a full factorial mesocosm experiment to examine the consequences of plastic hatching timing (early or late) for larval growth and survival, and metamorphic size and timing across larval environments that vary in resources and perceived or actual predation risk. In general, we hypothesize that hatching early reduces survival and has lasting effects on phenotypes, that both effects vary with the post-hatching environment, and that phenotypic effects attenuate with time. We also hypothesize that some effects of hatching timing are comparable in magnitude to those of other important environmental variables. We specifically predict that: (1) larval predators increase the survival cost of early hatching. (2) Early hatching increases larval growth rate. (3) Growth of early-hatched tadpoles suffers more from low resources than does that of late-hatched tadpoles, exacerbating higher predation on early-hatched individuals. (4) High resources will benefit early-hatched tadpoles more than late-hatched tadpoles.

(5) Most additional mortality of early-hatched animals occurs early in ontogeny.

METHODS

Red-eyed treefrogs are common in Neotropical wet forests from the Yucatan to Colombia (Duellman 2001). They deposit eggs on plants over ponds and tadpoles drop into the water upon hatching. At our field site in Gamboa, Panama, undisturbed eggs hatch 6–7 days post-oviposition, but can hatch as early as 4 days post-oviposition in response to egg-stage risks (Warkentin 2000b).

Our experiment was conducted in 96 400-L plastic mesocosms (0.7 m diameter base, 0.9 m diameter mouth, 0.8 m high, with screened drain holes at 0.75 m height) in a partially shaded field at the forest edge at the Smithsonian Tropical Research Institute. We manipulated three variables potentially important to *A. callidryas* survival and growth to metamorphosis: hatching age, resource level, and predation risk. Embryos were stimulated to hatch at either 4 or 6 days post-oviposition (early- and late-hatched). High or low levels of resources (1.5 or 0.75 g of Sera micron powder; Sera, Heinsberg, Germany) were added to each mesocosm every five days. We also manipulated the presence and lethality of *Pantala flavescens* (Odonata: Libellulidae) dragonfly naiads, a common tadpole predator at our site. Mesocosms contained either two free-roaming naiads (hereafter "lethal" or "L"), two separately caged naiads (hereafter "caged" or "NL," nonlethal), or were predator-free controls (hereafter "control" or "C"). Caged naiads were checked and fed two *A. callidryas* hatchlings three times per week throughout the experiment. Predators were replaced if they metamorphosed or died. The experiment lasted until all tadpoles died or metamorphosed.

Resource levels were chosen based on our prior work so that the low level caused competition for food. Predator density (5 naiads/m^3) was within the range found in ponds at our study site (0.4–11.1 naiads/m^3; J. Touchon and J. Vonesh, *unpublished data*). Initial tadpole density (100 tadpoles/m^3) was within the range of hatchling inputs to ponds and chosen to ensure that some tadpoles metamorphosed from the lethal-predator treatment.

We conducted a full factorial cross of the three variables for 12 treatment combinations (2 hatching ages × 2 resource levels × 3 predator treatments) set up in eight fully replicated spatial and temporal blocks ($N = 96$ experimental units). Mesocosms were filled 3–5 days before each block began with a mixture of captured rainwater and aged tap water and fitted with screen covers to prevent colonization by unwanted organisms. To promote a healthy aquatic community in each mesocosm, we added 250 g of leaf litter collected from the nearby Experimental Pond (9°7′14.88″ N, 79°42′14.11″ W) and a 1-L inoculate of zoo- and phytoplankton collected from Ocelot Pond (9°6′8.62″ N, 79°40′56.96″ W). To facilitate finding tadpoles and predators during censuses, 80% of the leaf litter (200 g) was contained in a screen bag. Due to variation in tree canopy above mesocosms, different blocks experienced different amounts of shading, but replicates within each block experienced similar shading.

For each block, we collected 20–25 *A. callidryas* egg masses from either Ocelot or Experimental Pond (191 clutches in total, ~40 eggs each) the morning after oviposition, 29 May–6 June 2009. We maintained clutches in an open-air laboratory, misted them regularly with aged tap water to maintain hydration, and randomly assigned one-half to each hatching treatment. When embryos were 4 or 6 days post-oviposition, as appropriate, they were manually stimulated to hatch at ca. 11:00 hours into a single container, allowing tadpoles to mix in the water. The first tadpoles (blocks 1–2 early-hatched) were added to mesocosms on 2 June. The last tadpoles (block 8 late-hatched) were added on 12 June and the experiment ended when the last metamorph emerged on 17 December.

We haphazardly drew groups of 50 hatchlings from the tadpoles for each block, digitally photographed them in a shallow tray with a ruler, and added them to each mesocosm immediately after hatching. To monitor tadpole growth and survival, we dipnetted all tadpoles out of each mesocosm 15 and 30 days after early hatching in their block (i.e., 19 and 34 days post-oviposition) and photographed them again. Tadpole total length (snout to tail tip) at hatching and at each census was measured from photographs using ImageJ digital image analysis software (Rasband 1997–2012).

Once tadpoles in a mesocosm were observed to have large hindlimbs, that mesocosm was checked each morning for any emerged metamorphs, which climbed out of the water and slept on the inner lip of the tank, under the screen lid. Metamorphs were brought to the open-air laboratory and housed individually in 266-mL cups with perforated lids to complete tail resorption. Cups contained a few milliliters of aged tap water to maintain metamorph hydration. We measured tail length and snout–vent length (SVL) at emergence and SVL and mass at tail resorption, then released froglets at their pond of origin.

Statistical analysis

Statistical analyses were conducted in R 2.10.1 (R Development Core Team 2009). We used generalized linear mixed models (GLMM) using the function lmer in the "lme4" package for all analyses (Bates and Maechler 2009). See Appendix for details of statistical methods, including the structure of fixed and random effects in all models, sample sizes, and post hoc analyses. Briefly, we always began by fitting the maximal model with all possible interactions of fixed effects and then compared increasingly simplified, nested models with likelihood ratio tests to estimate P values of factors and their interactions. Analyses of tadpole size and survival used

one datum per tank (means and counts), with "block" included as a random effect. Analyses of metamorph phenotype and emergence time used data from individuals, with block and "tank within block" as random effects. We used tadpole total length and metamorph SVL at tail resorption as measures of size.

When early hatchlings suffer higher mortality than late hatchlings (Warkentin 1995), effects of hatching age on growth could be driven by changes in density rather than directly by hatching timing. To disentangle direct effects of hatching age and those mediated by mortality, we tested for effects of hatching age and the number of surviving tadpoles per tank on tadpole size.

We present analyses of three aspects of metamorph phenotype: SVL at tail resorption, relative tail length at emergence (tail length/[SVL + tail length], an indicator of how long animals with forelimbs remain in the water), and the time needed to resorb the tail after emerging (i.e., reach Gosner stage 46; Gosner [1960]). Results of analyses of SVL at emergence and mass at tail resorption were similar to those for SVL at tail resorption and are not presented for brevity, and results of relative tail length and time to tail resorption are presented in the Appendix.

Results

Effects on larval growth and survival

Overall tadpole size was influenced by hatching age, resource level, and predator treatment (Fig. 1A–C; hatching age, $\chi^2 = 9.3$, $P = 0.002$; resources, $\chi^2 = 32.2$, $P < 0.00001$; predators, $\chi^2 = 21.4$, $P < 0.00001$). Unsurprisingly, there was a strong effect of time on tadpole size ($\chi^2 = 33.7$, $P < 0.00001$); tadpole total length increased over time in all treatments. However, there was also an interaction between time and predator treatment ($\chi^2 = 44.0$, $P < 0.00001$). Post hoc tests revealed no differences in size among predator treatments at 15 days, but by 30 days tadpoles with lethal predators were 19.6% and 16.9% larger, respectively, than tadpoles from control and caged predator tanks, which did not differ from one another (Fig. 1A; post hoc tests, 15 days, L-C, lethal vs. control, $P = 0.23$, L-NL, $P = 0.54$, NL-C, $P = 0.55$; 30 days, L-C, $P < 0.0001$, L-NL, $P < 0.0001$, NL-C, $P = 0.59$). Differences in tadpole size due to hatching timing and resources were most evident at 15 days, when early-hatched tadpoles were 3.4% larger than late-hatched animals (Fig. 1B) and tadpoles in high resources were 8.4% larger than those with low resources (Fig. 1C).

Effects on tadpole survival were somewhat similar to those for growth. Lethal predators had the strongest effects on tadpole survival, reducing it by >40% compared to control and caged-predator treatments (Fig. 1D; $\chi^2 = 209.6$, $P < 0.00001$; post hoc tests, L-C, $P < 0.0001$, L-NL, $P < 0.0001$, NL-C, $P = 0.86$). Hatching age and resource levels also affected tadpole survival, with 7.6% more tadpoles surviving in late-hatched treatments than early, and 4.1% more surviving in

high-resource treatments than low (Fig. 1E, F; hatching age, $\chi^2 = 19.6$, $P < 0.00001$; resource level, $\chi^2 = 6.9$, $P = 0.009$). There were no significant interactions between hatching age, predators, and resource level, nor did survival change significantly between the two censuses, indicating that most mortality occurred in the first 15 days.

The increase in size of tadpoles in the early-hatched treatment was not simply due to reduced density (Fig. 1B, E). The number of tadpoles alive in a tank strongly affected tadpole size, but hatching age had a significant effect even after accounting for variation in survival (density, $\chi^2 = 18.4$, $P < 0.00001$; hatching age, $\chi^2 = 4.4$, $P = 0.03$).

Effects on survival to metamorphosis

In total, 2493 metamorphs successfully emerged (52.0% survival overall). Survival to metamorphosis, as earlier in the larval period, was most strongly affected by lethal predators (Fig. 1G). Only 44% of tadpoles with lethal predators survived to metamorphosis, 30% fewer than with caged predators and 40% fewer than controls (Fig. 1G; $\chi^2 = 58.8$, $P < 0.00001$; post hoc tests, L-C, $P < 0.0001$, L-NL, $P < 0.0001$, NL-C, $P = 0.09$). Resources and hatching age also affected survival to metamorphosis, but to a lesser degree. Hatching early, at 4 days post-oviposition, caused a 9% decrease in survival to metamorphosis (Fig. 1H; $\chi^2 = 6.3$, $P = 0.01$). Similarly, low resources decreased survival by 9% compared to tadpoles in tanks with higher resources (Fig. 1I; $\chi^2 = 8.4$, $P = 0.003$). There were no significant interactions between hatching age, predators, and resources on survival to metamorphosis.

Effects on timing of metamorphosis and metamorph phenotype

Metamorphs emerged from tanks between 35 and 202 days post-oviposition. The age at which A. callidryas left the water was strongly affected by resource level and predator treatment (Fig. 2; resource, $\chi^2 = 31.7$, $P < 0.00001$; predator, $\chi^2 = 32.9$, $P < 0.00001$). Tadpoles in low resource tanks metamorphosed, on average, 34% later than those with high resources, and tadpoles with lethal predators metamorphosed 26% earlier than tadpoles with caged predators and 39% earlier than those in control tanks (Fig. 2). There was also a significant interaction between predator and resource treatments; the strength of the lethal predator effect differed under low and high resource conditions (Fig. 2; resource × predator, $\chi^2 = 7.1$, $P = 0.029$; post hoc tests, low resources, L-C, $P < 0.0001$, L-NL, $P = 0.0006$, NL-C, $P = 0.14$; high resources, L-C, $P = 0.007$, L-NL, $P = 0.11$, NL-C, $P = 0.67$). With high resources, tadpoles emerged from lethal-predator tanks 17% (~10 days) earlier than from controls or caged-predator tanks, whereas with low resources they emerged 33% (~30 days) earlier (Fig. 2). There was no detectable effect of hatching age on metamorphic timing ($\chi^2 = 1.0$, $P = 0.32$).
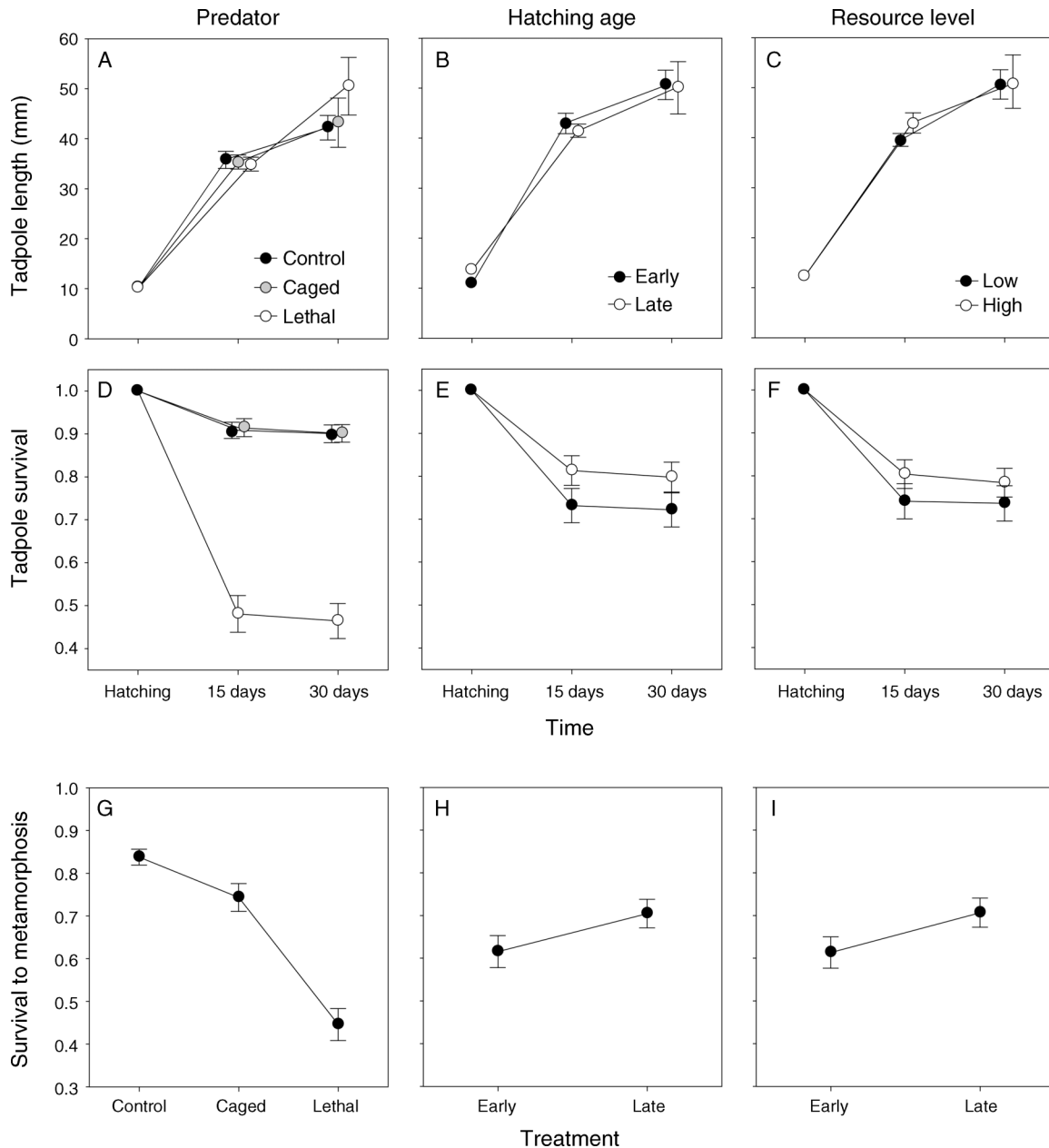
FIG. 1. Effects of predator (*Pantala flavescens*, dragonfly naiads), hatching age, and resource treatments on (A–C) larval size, (D–F) larval survival, and (G–I) survival to metamorphosis of the red-eyed treefrog, *Agalychnis callidryas*, in 400-L mesocosms in Gamboa, Panama. Predator treatments were a no-predator control, two caged dragonfly nymph predators, or two free-roaming lethal dragonfly nymph predators. Hatching ages were at 4 days post-oviposition or 6 days post-oviposition. High resources were twice that of low resources. Larval size and survival were measured at 15 and 30 days after the start of the experiment. Data are means and 95% confidence intervals of fitted values from mixed models accounting for block and time effects. Symbols in panels (A)–(D) are horizontally offset to increase visibility.

The size of metamorphs (SVL at tail resorption) was affected by predator treatment, resource level, age at metamorphosis, interactions between resources and age at metamorphosis and between hatching age and age at metamorphosis, and the three-way interaction between age at metamorphosis, resources, and predator treatment (Fig. 3; predator, $\chi^2 = 60.5$, $P < 0.00001$; resources, $\chi^2 = 26.0$, $P < 0.00001$; age at metamorphosis, $\chi^2 = 1376.3$, $P < 0.00001$; hatching age $\times$ age, $\chi^2 = 5.2$, $P = 0.02$; resources $\times$ age, $\chi^2 = 196.8$, $P < 0.00001$; predator $\times$ resources $\times$ age, $\chi^2 = 17.1$, $P = 0.0002$). In essence, the three-way interaction indicates that the interacting effects of resources and predators changed over the 5.5-month period of metamorph emergence
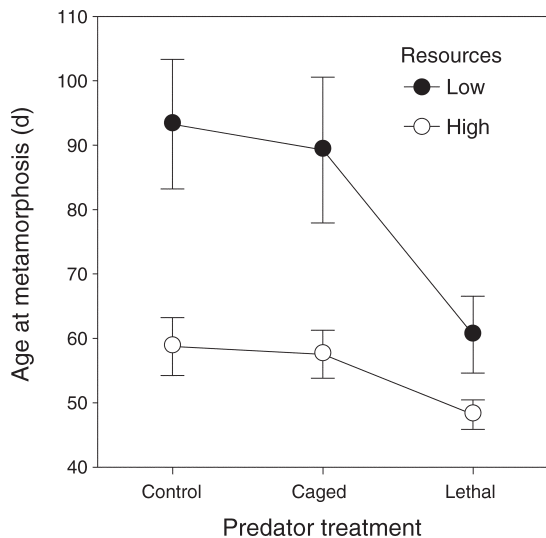
FIG. 2. The interaction between resource variation and predator treatment on timing of metamorphosis of *Agalychnis callidryas* tadpoles (days from oviposition to emergence from the water). Data are means and 95% confidence intervals of fitted values from mixed models accounting for block and tank effects.

(Fig. 3A–C). Under low resource conditions, the increase in size over time did not differ among predator treatments and only metamorphs from control tanks were smaller than those from tanks with lethal predators (Fig. 3A–C solid lines; post hoc tests, intercept, L-C, P < 0.0001, L-NL, P < 0.0001, NL-C, $P = 0.15$; slope, L-C, $P = 0.75$, L-NL, $P = 0.41$, NL-C, $P = 0.50$). However, under high resource conditions, the initial size of metamorphs from lethal-predator tanks was larger than those from control and caged-predator tanks, but as the emergence progressed they increased less in size (Fig. 3A–C dashed lines; post hoc tests, intercept, L-C, P < 0.0001, L-NL, P < 0.0001, NL-C, $P = 0.26$; slope, L-C, P < 0.0001, L-NL, $P = 0.0002$, NL-C, $P = 0.96$).

Beyond these strong effects of predators and resources, we detected significant effects of hatching age on size (Fig. 3D). Early in the emergence, late-hatched metamorphs were smaller than early-hatched ones, but their size increased more as the emergence progressed, converging on that of early-hatched animals.

The relative tail length of early-hatched metamorphs was 2% greater than for metamorphs that had hatched late (Appendix: Fig. A2; $\chi^2 = 4.8$, $P = 0.028$; see Appendix for other treatment effects on tail length). Tail length at emergence was also influenced by the interaction between resources and predator treatment (see additional results in Appendix). The time meta-
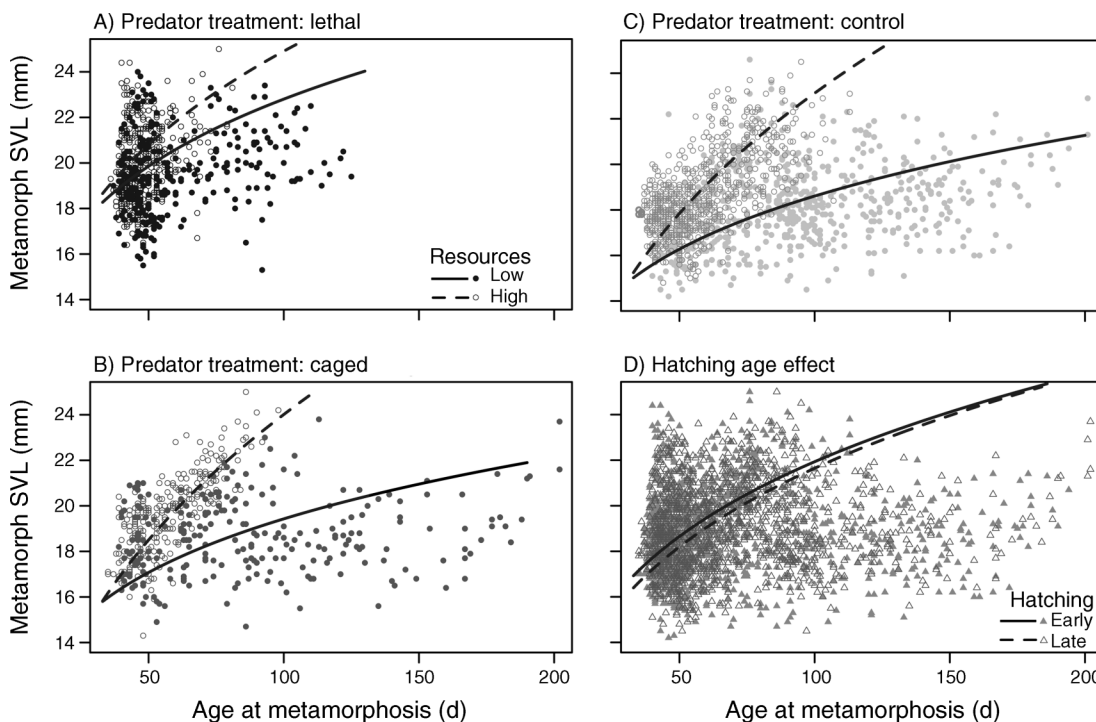

FIG. 3. The relationship between *Agalychnis callidryas* size and age at metamorphosis with predator, hatching age, and resource treatments. (A–C) Metamorph snout–vent length (SVL) at tail resorption was affected by interactions between resource level, predator treatment, and age at metamorphosis. (D) Metamorph size was also affected by hatching age. Regression lines are calculated from coefficients from mixed models accounting for block and tank effects. Individual points are faded to increase clarity of regression lines. Lines in panel (D) deviate from the data after ca. day 100 due to high leverage of data before that point.

morphs took to fully resorb their tail after emergence was strongly influenced by relative tail length (Appendix: Fig. A4; $\chi^2 = 591.2$, $P < 0.00001$); relatively longer tails took longer to resorb (Appendix: Fig. A4). However, there was also an effect of larval resources, with metamorphs from low-resource tanks taking longer to resorb their tail than those from high-resource tanks (Appendix: Fig. A4; $\chi^2 = 19.0$, $P = 0.00001$).

DISCUSSION

Environmentally cued hatching in animals is seen throughout bilateria (reviewed in Warkentin 2011a). Embryos adaptively alter their timing of hatching in response to variation in egg-stage risks such as predators and pathogens, and cues of larval-stage predators, resources, and conspecifics (Warkentin 2011a, b). Moreover, direct effects on embryonic development can also alter hatchling phenotypes (Orizaola et al. 2010, Touchon and Warkentin 2010). Plastic responses to environmental variation in the egg stage may carry over to affect phenotypes and performance or alter responses to environmental conditions later in life. Such long-term effects of egg environments may be common in nature, but have only recently begun to be investigated.

Here, we demonstrate that the hatching timing of *A. callidryas* embryos, which varies plastically with threats to eggs, has effects that persist through the larval period to alter survivorship and phenotypes at metamorphosis, and that these effects are detectable across strong numerical and phenotypic effects of predators and resources. Moreover, effects of hatching timing can be of equivalent or greater magnitude than those of resource level, long recognized as a factor structuring predation and competitive interactions. Such lasting consequences of embryo responses to their environment have implications for how ecologists think about events early in life and the effects of plasticity on fundamental processes such as growth and recruitment. If developmental plasticity of early life stages is as ubiquitous as it seems (West-Eberhard 2003, Warkentin 2011a), it is important to understand long-term effects of early plastic responses.

Lasting and latent effects of hatching age

The largest lasting effect of hatching timing was on survival. Hatching two days prematurely reduced survival by 7.6% after 15 days, and by 9% at metamorphosis (Fig. 1). Contrary to our predictions, this effect was independent of both predator exposure and resource level and did not attenuate over the larval period. It appeared to stem from a general reduction in tadpole viability both shortly after hatching and also closer to metamorphosis, as there was little tadpole mortality between 15 and 30 days. The viability cost of early hatching that we detected here adds another source of selection against unnecessary premature hatching. Reduced viability may result from stresses of the early hatching process or from physiological demands of the post-hatching environment for which early hatchlings are less prepared. Parallel effects are seen in mammals, where premature birth can be a major cause of neonate mortality and morbidity (Beck et al. 2010, Teune et al. 2011).

A second lasting effect of hatching timing was on growth. During the larval period, early-hatched *A. callidryas* were larger than late-hatched animals at 19 days post-oviposition, despite being smaller at hatching (Fig. 1B). The same was true for metamorphs early in the period of emergence (Fig. 3D). This result appears different from the growth and size advantage amphibian larvae receive when hatching from larger eggs, where larger eggs lead to larger hatchlings that have greater survival through the larval period (Semlitsch and Gibbons 1990, Kaplan 1992). The effect of hatching timing we found was not simply due to the size at which animals entered the water; early hatching changed the subsequent growth rate. This altered growth rate might stem from either physiological or behavioral changes. Rapid growth can entail physiological costs (Metcalfe and Monaghan 2001, Mangel and Munch 2005), and faster growth of early-hatched tadpoles may contribute to their lower viability.

A size advantage of early-hatched tadpoles at metamorphosis was not detected in a previous, smaller, study (Warkentin 1999b). Nonetheless, faster larval growth and larger size at metamorphosis likely confer benefits that may partially compensate for some of the costs of early hatching. In a number of amphibian species, greater size at metamorphosis is associated with enhanced jumping performance (Tejedo et al. 2000, Gomez-Mestre et al. 2010), age and size at first reproduction (Scott 1994, Altwegg 2003, Berven 2009), and survival (Altwegg 2003). It may also affect froglet predation. In an African reed frog, *Hyperolius spinigularis*, larger metamorphs are less vulnerable to fishing spiders (Vonesh 2005b); however, in that species early hatchlings develop into smaller, not larger, metamorphs (Vonesh and Bolker 2005).

A third long-delayed or latent effect of hatching age, although of smaller magnitude, was on tail length at metamorph emergence; early-hatched animals left the water with longer tails than did late-hatched individuals (Appendix: Fig. A2). Differences in tail length when froglets leave the water reflect the time since forelimb emergence (Walsh et al. 2008). Furthermore, the period from forelimb emergence through dispersal from the pond is particularly dangerous, both in the water and on land (J. C. Touchon, R. R. Jiménez, S. H. Abinette, J. R. Vonesh, and K. M. Warkentin, *unpublished manuscript*). In water, forelimb emergence increases drag and reduces swimming performance (Huey 1980), whereas a longer tail on land decreases jumping ability and increases the probability of capture (Wassersug and Sperry 1977, Arnold and Wassersug 1978). Thus, altering the timing of emergence from the water, relative to tail resorption,

changes the balance of risks and may impact froglet survival.

We found no evidence that hatching timing affected the timing of metamorphosis in any larval environment we tested, congruent with Capellán and Nicieza (2007). Animals that entered the water two days early emerged as metamorphs at the same time as those that hatched later. In contrast, resource levels and lethal predators had strong effects on the timing of metamorphosis.

Lasting effects of larval predators and resources

Predation and competition, long recognized as important drivers of life histories (Sih et al. 1985, Gurevitch et al. 2000), had strong effects on *A. callidryas* survival to and phenotype at metamorphosis. Lethal predators caused a 40% reduction in survival compared to control treatments, with most of that mortality occurring in the first 15 days. Although the initial effect of predators on tadpole growth was negative, this early thinning increased per capita resource availability and substantially improved later growth, after tadpoles reached less vulnerable sizes (McCoy et al. 2011), resulting in larger metamorphs from lethal-predator tanks. Nonconsumptive effects of predators on survival were substantially weaker than consumptive effects, and similar in magnitude to effects of hatching age. In comparison to control tanks, caged predators reduced survival to metamorphosis by 10%. However, this mortality occurred relatively late in the larval period (Fig. 1D, G), which may account for the relatively small increase in metamorph size in caged-predator treatments, relative to controls (Fig. 3B, C).

Many effects of predators and resources on the larval period and metamorph phenotype were context dependent. Lethal predators always reduced the larval period, relative to control or caged-predator treatments; however, this effect was twice as strong under low resource conditions (Fig. 2). Conversely, high resources always reduced the larval period, but this effect was weakened by the presence of lethal predators. For metamorph size, effects of predator and resource treatments, like those of hatching age, varied across the emergence period (Fig. 3A–C). For animals that emerged early, resource effects were weak and predator effects strong; thinning increased size. As the period of emergence progressed, resource level became a stronger determinant of metamorph size. This may be because as animals left the mesocosms, more resources per capita were available for remaining tadpoles. Such effects are likely to occur in nature at the end of the breeding season. Similar complex relationships between embryo phenotype and larval environment exist in wood frogs (*Rana sylvatica*), where density and resource level interact with egg diameter to affect size at metamorphosis and length of the larval period (Berven and Chadra 1988).

Effects of predators and resource levels on tail length at emergence were also contextual and varied across the emergence period (see additional results in Appendix).

For instance, with low resources tail length at emergence was unaffected by predator treatment. However, with high resources, tail length varied across the emergence period differently for froglets raised with lethal predators or with caged predators (Appendix: Fig. A3).

Cascading effects of hatching plasticity

Our data offer a starting point to consider how plasticity in hatching timing may have larger scale, population level effects. Morphological and behavioral plasticity are known to alter community trophic links and stabilize population dynamics in a variety of organisms (Werner and Peacor 2003, Kishida et al. 2010). Although our study only examined the effects of hatching plasticity on interactions with a single predator, we may expect induced changes in hatching timing to alter *Agalychnis*–resource relationships as well as competitive interactions with other anuran larvae (Gonzalez et al. 2011). The viability cost of early hatching will add effects of changing density to those of altered phenotypes. Spatial or temporal variation in egg predator abundance (Hite 2009) will change the relative abundance of induced and spontaneously hatched individuals entering ponds, with cascading effects on larval growth and interactions with predators before and after metamorphosis. Furthermore, the lasting effects of plastic hatching timing on metamorph phenotype are likely to affect vulnerability to predators as froglets leave the pond (Arnold and Wassersug 1978; J. C. Touchon, R. R. Jiménez, S. H. Abinette, J. R. Vonesh, and K. M. Warkentin, *unpublished manuscript*).

Why does hatching timing have persistent effects?

Hatching represents a dramatic change in the embryo's developmental environment, altering physical conditions, resources, and natural enemies. Despite its small absolute magnitude compared to the 167-day range in larval period we observed, a two-day acceleration of hatching shortens *A. callidryas*' embryonic period by ~30%. This means hatchlings enter the water with different morphology, physiology, and behavioral abilities (Warkentin 1999b, 2000a). The immediate effects of hatching age presumably stem directly from these developmental differences among hatchlings that alter their interactions with aquatic predators (Warkentin 1999a) and their viability (Fig. 1). These dramatic morphological differences are, however, transient (Warkentin 1999b).

The faster growth of early-hatched tadpoles that we observed was not simply due to thinning. It is consistent with growth patterns of *H. spinigularis* that hatch early in response to egg predators (Vonesh 2005a) and with the compensatory growth of *Rana arvalis* that hatch late and small due to cold (Orizaola et al. 2010). Compensatory acceleration of growth of small hatchlings or neonates occurs in insects (De Block and Stoks 2005), birds (Benowitz-Fredericks and Kitaysky 2005), and mammals (Euser et al. 2008), in addition to amphibians

(Semlitsch and Caldwell 1982, Orizaola et al. 2010, Touchon and Warkentin 2010), and thus appears widespread. When predation is size dependent, fast growth can reduce mortality, partially compensating for earlier exposure to larval predators at an initially smaller size (Werner and Gilliam 1984, Vonesh and Bolker 2005, McCoy et al. 2011). However, faster growth may also impose physiological costs (Metcalfe and Monaghan 2001, Mangel and Munch 2005), which are part of a suite of selective factors that make submaximal growth rates common in nature. Such costs may contribute to the higher mortality of early-hatched *A. callidryas* tadpoles.

It is also possible that the physiological and developmental mechanisms by which embryos actively accelerate or, for other species, delay hatching in response to environmental cues have lasting effects. Although these mechanisms are largely unknown for hatching (but see Weiss et al. 2007), they have been well studied for metamorphosis. For instance, plastic shifts in metamorphic timing involve glucocorticoid stress hormones, which can affect subsequent development or function (Denver 1997). Glucocorticoid exposure at critical points in development can alter neural circuitry (Hu et al. 2008), with potential lasting consequences for behavior, morphology, and fitness. In addition, exposure to the larval environment earlier, or egg environment for longer, might affect the trajectory of subsequent development. Identifying specific mechanisms involved in regulating hatching timing will help elucidate their potential contribution to lasting effects on phenotypes.

Conclusions

Plasticity in hatching timing is widespread (Warkentin 2011a, b) and likely important in ways currently underappreciated by ecologists. In particular, it appears that the effects of induced shifts in hatching timing may vary among species or contexts (Warkentin 1999a, Vonesh and Osenberg 2003, Vonesh and Bolker 2005, Capellán and Nicieza 2007). Indeed, we found that the relationship between hatching timing and metamorph phenotype changed with larval period, highlighting how context may alter effects of early events on subsequent phenotypes. Ultimately, embryos that hatched early suffered greater mortality through the larval period than did those that hatched late, but were larger at metamorphosis and emerged from the water with longer tails. Importantly, effects on size were not purely due to thinning, but were directly affected by hatching timing itself. The phenotype with which amphibians leave their pond is an important predictor of post-metamorphic survival and can affect reproductive success (Semlitsch et al. 1988, Scott 1994, Altwegg and Reyer 2003). Both numerical and phenotypic effects of hatching timing persisted through the larval period and were detectable at metamorphosis, on top of strong effects of larval predators and resources. The plastic responses of embryos to their variable egg environments have implications for both population processes and the fates of individuals across multiple life stages.

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SUPPLEMENTAL MATERIAL

Appendix

Details on statistical methods with appropriate references, results of analyses of variation in tail length at metamorph emergence, a table detailing the structure of all mixed models used in the paper, and four figures showing variation in metamorph tail length, how tail length at emergence was affected by interactions between age at metamorphosis and hatching age and predator treatment, and how larval resources affected the amount of time metamorphs needed to resorb the tail after emergence (*Ecological Archives* E094-073-A1).