

Environmental context shapes immediate and cumulative costs of risk-induced early hatching

B. Willink · M. S. Palmer · T. Landberg · J. R. Vonesh ·
K. M. Warkentin

Received: 22 January 2013 / Accepted: 14 June 2013 / Published online: 4 July 2013
© Springer Science+Business Media Dordrecht 2013

Abstract In animals with complex life cycles, fitness trade-offs across life stages determine the optimal time for transitions between stages. If these trade-offs vary predictably, adaptive plasticity in the timing of life history transitions may evolve. For instance, embryos of many species are capable of accelerating hatching to escape from egg predation and other hazards, but for plasticity in hatching timing to be selectively maintained, early hatching must also entail costs, probably in subsequent life stages. However the post-hatching environment, which influences this cost, is variable in nature. We assessed how two elements of the post-hatching environment, predator species and age

Electronic supplementary material The online version of this article (doi:[10.1007/s10682-013-9661-z](https://doi.org/10.1007/s10682-013-9661-z)) contains supplementary material, which is available to authorized users.

B. Willink (✉)
Escuela de Biología, Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio,
2060 San José, Costa Rica
e-mail: beatriz.willink@ucr.ac.cr

M. S. Palmer
Department of Zoology, Ohio Wesleyan University, 61 South Sandusky Street,
Delaware, OH 43015, USA

T. Landberg · K. M. Warkentin
Department of Biology, Boston University, 5 Cummington Mall, Boston, MA 02215, USA

Present Address:
T. Landberg
Murray State University, Murray, KY, USA

J. R. Vonesh
Department of Biology, Virginia Commonwealth University,
1000 West Cary Street, Richmond, VA 23284, USA

K. M. Warkentin
Smithsonian Tropical Research Institute, Apartado Postal 0843-03092,
Panama, Republic of Panama

structure created by hatching age plasticity, affect costs of hatching early in red-eyed treefrogs, *Agalychnis callidryas*. Red-eyed treefrog embryos were induced to hatch at the onset of hatching competence or near the peak of spontaneous hatching and exposed to one of three insect predators in single or mixed hatching-age treatments. Age structure created by hatching-age plasticity did not affect tadpole survivorship or growth; however, the consequences of hatching timing depended on predator species and foraging mode. Tadpoles that were induced to hatch early experienced initially higher mortality rates only with the more actively foraging predator. Nonetheless, mortality costs of accelerated hatching were apparent with all predators once we factored in the longer duration of exposure that early hatchlings experience in nature. This study suggests that extended exposure of young larvae to predators may be a general cost of early hatching, explaining why spontaneous hatching occurs later in life across variable environmental contexts.

Keywords Complex life cycle · Life history switch point · Environmental variability · Growth · Phenotypic plasticity · Trade-off

Introduction

Adaptive phenotypic plasticity can evolve when environmental conditions predictably affect fitness, so that phenotype-environment match is enhanced by producing alternative phenotypes in different environments (Moran 1992). In animals with complex life cycles, the optimal time of life history transitions depends on fitness trade-offs across stages (Werner 1986), and plasticity may evolve because risks and opportunities at each stage vary among environments. For instance, when stage-specific mortality risks are immediate it is almost certainly adaptive to escape them by accelerating the transition to the next life stage. However, this early transition is expected to bring fitness costs in subsequent stages for such plasticity to be selectively maintained.

Hatching is the first life history switch point in the life of most animals, and evidence for the ubiquity of plasticity in hatching timing is rapidly accumulating across animal taxa (reviewed in Warkentin 2011a). A variety of organisms can accelerate hatching to escape attacks by egg predators or pathogens (Warkentin 1995; Li 2002; Moreira and Barata 2005; Wedekind 2002; Touchon et al. 2006; Ireland et al. 2007; Doody and Paull 2013). However, most studies addressing the evolution of plasticity in life history transitions focus on metamorphosis and the onset of reproduction. We know little about the general mechanisms that maintain plasticity in the timing of hatching (e.g. Gomez-Mestre et al. 2008a; Warkentin 2011b), and even less about the role of the environmental context shaping the magnitude and form of the trade-offs associated with hatching timing.

To date, most studies assessing the costs of risk-induced hatching show that early hatchlings experience higher predation (Warkentin 1995, 1999a; Gomez-Mestre et al. 2008a). These studies have measured instantaneous rates of mortality that depend on hatching phenotype, but have not factored in the time over which such rates apply. Predation rates are often critically high during early ontogeny (Werner and Gilliam 1984; Ireland 1989; Tanhuanpää et al. 2001), thus decisions in response to risk during this period (i.e. when to hatch) probably have important fitness consequences. For instance, for red-eyed treefrogs, *Agalychnis callidryas*, predation declines rapidly as tadpoles grow (McCoy et al. 2011). Although their larval period may last months, most of larval mortality can occur in the first 2 weeks (Touchon et al. 2013). Plasticity in hatching timing generates variation in how much of early development is spent in the egg versus the larval stage.

Specifically, hatching early adds time to the beginning of the larval period, when mortality rates are high. An animal’s phenotype at any time point affects its current risk of predation, but fitness ultimately depends on survival over time. Therefore, effects of both hatchling phenotypes and the partitioning of time between life stages with different risks will contribute to costs of early hatching. However, earlier entry into the larval environment might also allow for compensatory growth to reduce cumulative mortality (e.g. Vonesh and Bolker 2005), potentially mitigating costs. Understanding these effects is further complicated by the fact that, in nature, embryos of most species hatch into variable environmental contexts, with diverse predators and alternative prey. Here, we investigated phenotypic and time-partitioning effects of predator-induced accelerated hatching in *A. callidryas*. Specifically, we asked how two components of the post-hatching environment, prey age-structure and predator identity, affect the mortality and growth of early and late hatchlings.

The arboreal embryos of *A. callidryas* can hatch up to 30 % prematurely to escape a variety of threats to eggs, including predator attacks, flooding, and pathogen infection (Fig. 1; Warkentin 1995, 2000, 2002; Warkentin et al. 2001). At some ponds predators attack over half of clutches laid (Warkentin 1995, 2000), thus induced early hatching is frequent. Red-eyed treefrogs breed throughout the Neotropical rainy season, whenever it rains, so clutches of different ages are typically present at ponds concurrently (Warkentin et al. 2006). Embryos of different egg cohorts may hatch at the same time if, for instance, the younger embryos are attacked by an egg predator while the older eggs hatch spontaneously. Thus, plasticity in hatching timing contributes to population structure by altering the age, size and stage at which animals enter the larval environment (hereafter, age structure).

Previous studies aiming to quantify fitness costs of early hatching have measured predation risk for early and late-hatched larvae separately (e.g. Warkentin 1995; Gomez-Mestre and Warkentin 2007). We assessed how the age structure of hatchlings entering the pond together shapes the consequences of early hatching. We hypothesized that even if early hatchlings are more vulnerable to predators, the presence of alternative prey could reduce their predation rate if predators preferentially attack larger prey that may grant

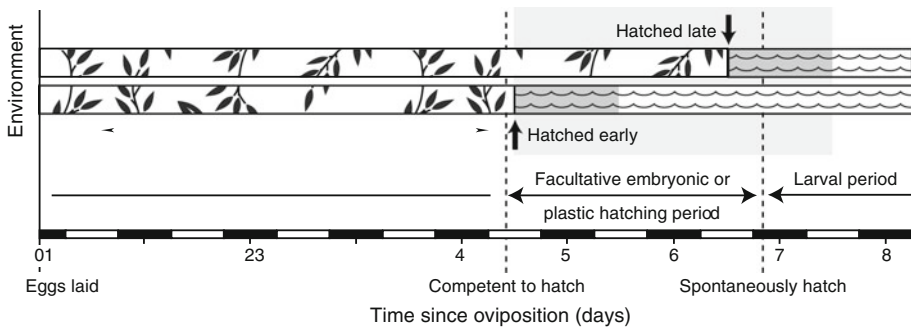


Fig. 1 Timeline of critical events in the early ontogeny of *A. callidryas*, based on development rates in Gamboa, Panama. All embryos begin development in arboreal eggs (*leaf pattern*) and eggs are laid at night (*black bars*), most between 2200 and 0200 hours. Embryos become capable of hatching during the day at age 4 days, entering a period of facultative embryonic development during which they hatch in response to egg predators, pathogens, and other threats. Spontaneous hatching peaks at age 6 days, shortly after dark. Upon hatching, tadpoles enter the water (*wave pattern*) and are exposed to aquatic predators. We compared effects of hatching age on survival in two ways: (1) over their first 24 h in the water (*dark grey*) for tadpoles that hatched early and late in the plastic hatching period (*vertical arrows*), and (2) over the same 72 h period (*light grey*) for early hatchlings that spent the entire period in the water and late hatchlings that began in the egg and later entered the water

superior returns per unit of effort (e.g. Chrzanowski and Simek 1990). Thus, age structure may lessen the mortality costs of early hatching. Conversely, if predators prefer smaller or less developed prey, the presence of early hatchlings could enhance survivorship of larger or more developed prey (e.g. de Roos et al. 2003).

After hatching, red-eyed treefrogs are exposed to a new suite of potential predators. The effects of early hatching likely depend on predator identity, because predator foraging mode influences selection of prey that differ in motility and size (Greene 1986). Active foragers normally encounter and attack more sedentary prey than do ambush predators (Huey and Pianka 1981; Grant and Noakes 1987) and should pose a major threat to early hatchlings, which are initially less active than spontaneously hatched tadpoles (Warkentin 1999a; Gomez-Mestre et al. 2006). Also, relatively large predators that will consume many prey before satiation are expected to search more actively than relatively small predators (Rosenheim and Corbett 2003), and thus encounter inactive prey more often. In contrast, the inactivity of early hatchlings may protect them against ambush predators that rely heavily on prey movement to encounter prey. We exposed early and late-hatched tadpoles to three aquatic insects that differ in foraging behavior and predicted that costs of early hatching would be greater with predators that forage more actively.

Aquatic organisms are known to exhibit reduced growth in response to cues of predation risk (reviewed in Preisser et al. 2007). We hypothesized that if environmental context influenced mortality costs of early hatching it would also modulate growth. Early hatchlings can grow faster than conspecifics remaining in the egg because hatching releases them from physiological constraints on metabolism and development rate imposed by the egg capsule (Warkentin 1999b; Rogge and Warkentin 2008). However, we expected that growth of early hatchlings would be suppressed in environments that pose greater risks, such as with actively foraging predators, potentially overriding any growth acceleration from physiological release.

Finally, within a single egg cohort (i.e. eggs laid on the same night), some clutches are induced to hatch early while others remain undisturbed until they hatch spontaneously, thereby entirely avoiding aquatic predators for a longer period of early development (Fig. 1). Early hatchlings continue to develop rapidly during their additional time in the water and may become increasingly vulnerable to ambush predators. Here, we tested the hypothesis that the increased duration of exposure to aquatic predators overrides any immediate benefit of initial inactivity, so that when accounting for this time-partitioning component early hatching is costly regardless of predator foraging mode. Together, our results clarify the role of environmental context in shaping the mechanisms that maintain plasticity in the timing of a fundamental life history transition.

Materials and methods

Animal collection and handling

This research was conducted from June–July 2010 and June 2012 at the Smithsonian Tropical Research Institute in Gamboa, Panama. Young *A. callidryas* egg clutches were collected from the Experimental Pond (9°7'14.88"N, 79°42'0.11"W) no more than 2 days post-oviposition. Clutches were maintained at ambient temperature and humidity in an open-air laboratory and misted regularly to prevent dehydration. For each experiment, hatching was induced 4 days post-oviposition, the onset of hatching competence (early hatchlings) or 48 h later, at 6 days post-oviposition, near the peak of spontaneous hatching

(late hatchlings, Fig. 1) by manually stimulating eggs and submerging them in water. To determine the onset of hatching competence, we tried to induce hatching of a few clutches starting at mid-day, 4 days post-oviposition. If embryos did not hatch readily further attempts were made at half-hour intervals. All embryos used in the experiments hatched before 1430 hours.

We assessed tadpole vulnerability to three insect predators that differ in their foraging behavior: one ambush predator and two that use active foraging, one small and one large. Aeshnid dragonfly nymphs (*Anax* spp, mean total length = 36.14 mm, SD = 4.81) are visually oriented (Sherk 1977) ambush predators regardless of habitat structural complexity (Michel and Adams 2009). They typically perch underwater on or under vegetation and leaf litter and strike when prey come nearby (Pritchard 1965). Libellulid dragonfly larvae (*Pantala flavescens*, mean total length = 17.31 mm, SD = 2.85) are small and less voracious predators (J. Vonesh & J. Touchon unpublished data). They can forage actively, propelling themselves through the water column with their ventilator chamber (Sherratt and Harvey 1989). Belostomatid giant water bugs (*Belostoma* cf. *porteri*, mean total length = 32.04 mm, SD = 3.82) can use both ambush and active strategies, but in venues with relatively simple structure, such as our experimental tanks, they rely more on active foraging (Cloarec 1990). Predators were collected from *A. callidryas* breeding ponds in Gambia where they are common and housed individually at ambient temperature and photoperiod. Prior to experiments predators were starved for 2 days to standardize hunger levels.

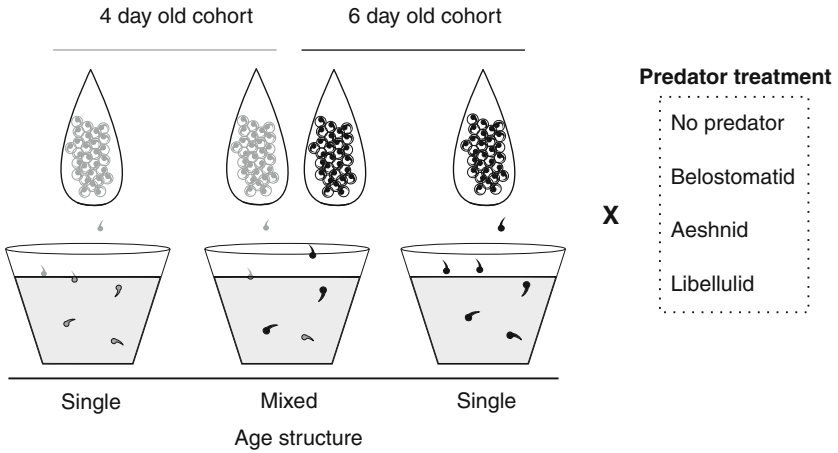
Experiment 1: Effects of hatchling phenotype and age structure on predation and growth rates

In this experiment we assessed effects of the phenotypes of early and late-hatched tadpoles on their initial mortality and growth rates, and asked how these effects are shaped by variation in predator identity and hatchling age structure. We measured predation and growth rates of early and late-hatched tadpoles over 24 h, both alone and in the presence of the other hatching age class. We used the same design in four sequential experiments, one with each predator type (belostomatid, aeshnid and libellulid) and a no predator control (Fig. 2a). We used a substitutive design with three treatments: 20 early hatchlings alone, 20 late hatchlings alone, and a mix of 10 early and 10 late hatchlings. Each treatment was replicated 10 times except for the belostomatid experiment that had 10, 9 and 11 replicates, respectively. Egg clutches laid 2 days apart were used in these experiments, so that 4 and 6 day old embryos could be induced to hatch on the same day.

Hatchlings from 10 to 15 clutches of each age were pooled and samples of 10 or 20 individuals drawn haphazardly. Groups of tadpoles were digitally photographed in dorsal view with a ruler and then placed into a randomized experimental array consisting of 30 round 60 L tubs with mesh covers. Tubs contained 40 L of aged tap water, 30 leaves to provide structure, 0.4 mg/L of Sera micron (Sera, Heisenberg, Germany) as food for tadpoles, and a single predator (or no predator for controls).

After 24 h, predators and tadpoles were removed from the experimental array. Survivors were photographed again and counted. Tadpoles from the mixed treatment were then examined individually under a dissecting microscope to determine their age class. To classify these tadpoles we used a combination of morphological traits that readily differentiated tadpoles from the early and late-hatched treatments (Fig. 3). Tadpole total lengths were measured from photographs using ImageJ (NIH; <http://rsbweb.nih.gov/ij/>). In photographs from the mixed treatment, tadpoles were identified as early or late-hatched as in

(a) Experiment 1: design for effects of hatchling phenotype and age structure



(b) Experiment 2: design for effects of the partitioning of early development across egg and larval stages

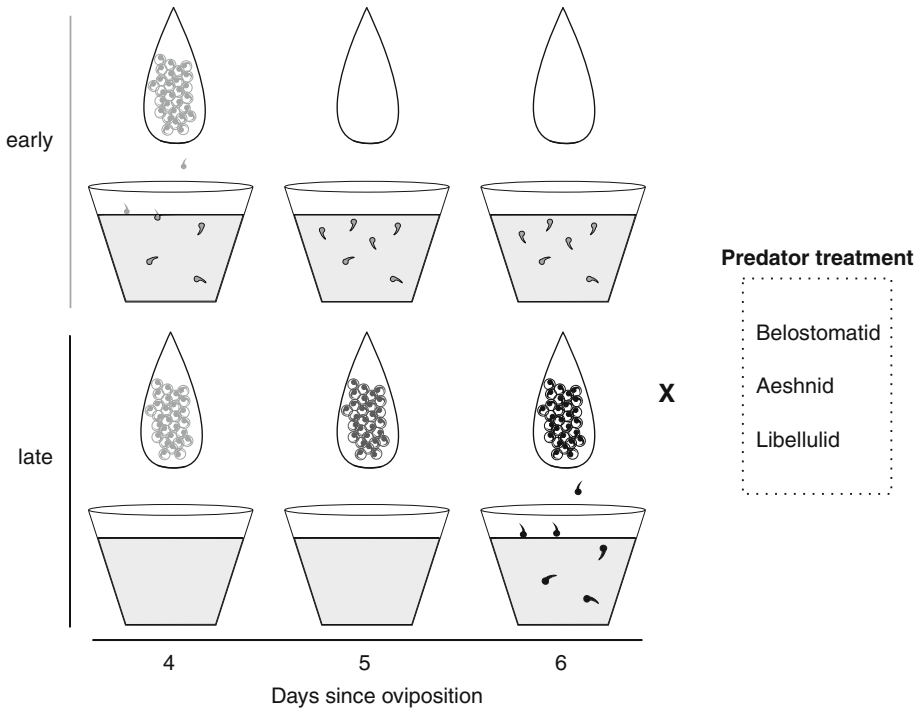


Fig. 2 Experimental design for testing **a** the phenotypic effects of early hatching in environments with variable prey age-structure and predator regimes and **b** the combined effects of hatchling phenotypes and the partitioning of early development across terrestrial egg and aquatic larval stages with three different predators. The experimental design was repeated in consecutive experiments with each predator in both experiments and with a predator-free control in **a**

Fig. 3; 8 % were not measured because their position or local image quality (shadows or glare) did not allow age class assignment. Average growth per replicate was calculated as the difference between average total length of tadpoles in initial and final photographs.

Experiment 2: Effects of hatching timing on survival through early ontogeny

To quantify the effect of extended exposure to aquatic predators due to early hatching (i.e. altered time partitioning), we assessed tadpole mortality at daily intervals over the 72 h period from the onset of hatching competence to 1 day after the peak of spontaneous hatching (age 4–7 days, Fig. 2b). Essentially all embryos that are healthy and developing normally at the onset of hatching competence survive to hatch spontaneously, if they are not disturbed by egg predators or other threats that induce hatching (Warkentin 2000; Gomez-Mestre and Warkentin 2007). Thus, our comparison of survivorship to 7 days post-oviposition specifically addresses the short-term effect of different durations of exposure to aquatic predators between tadpoles that were undisturbed as eggs and tadpoles that were threatened and induced to hatch at the onset of hatching competence. We conducted sequential experiments with each of the three predators above.

For each experiment, we collected egg clutches laid on a single night. When eggs were 4 days old, we randomly selected approximately half of the clutches and induced hatching

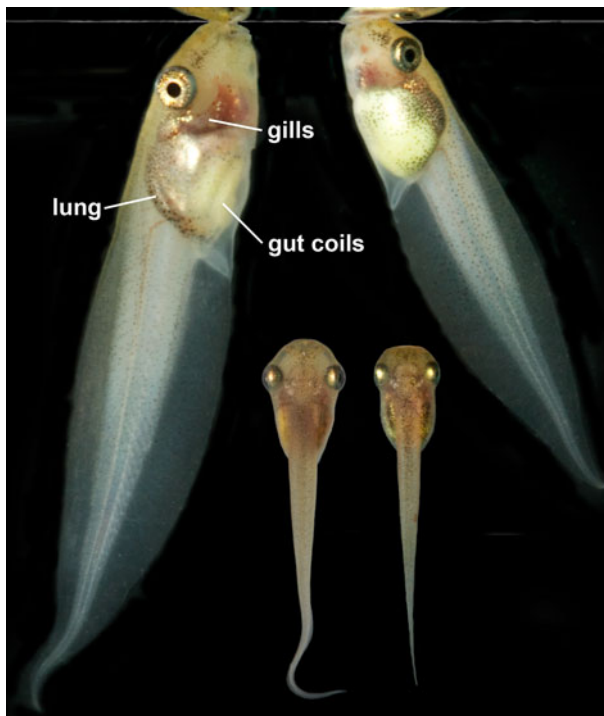


Fig. 3 Red-eyed treefrog tadpoles hatched at age 6 day (*left*) and 4 day (*right*) photographed together ~24 h after hatching. 1 day post-hatching, compared to early hatched tadpoles, late-hatched tadpoles have longer tails, both absolutely and relative to body length. Their snouts are wider and snout length clearly exceeds eye diameter. Their lungs and internal gills are larger, and gut coils are evident. In dorsal view (*insets*) they are widest at the eyes and taper posteriorly, whereas early hatched tadpoles still have bulbous yolk sacs

as above. The early hatchlings were pooled across clutches, haphazardly drawn from the pool, photographed in their groups, and placed into an array identical to that used in the 24-h experiments. 2 days (48 h) later the remaining clutches were induced to hatch and late hatchlings were added to the experiment. There were 20 tadpoles per replicate, 10 replicates per treatment (early and late hatched) for each predator, and 11–19 clutches pooled for each set of 10 replicates. For this experiment we ran 12 ‘no predator’ controls (9 with early hatchlings and 3 with late hatchlings). In these controls only early hatchlings (five of a single tank, 2.8 %) suffered mortality. We did not include a mixed treatment because in pilot experiments we could not accurately determine hatching age from external morphology for 7 day old tadpoles from a single egg cohort. We counted survivors every 24 h and ended experiments at 72 h. In both sets of experiments all predators and surviving tadpoles were released at their ponds of origin after the experiment was completed.

Data analysis

Tadpole survivorship was analyzed using generalized linear models (GLMs) with binomial error distribution and logit link function. Statistical significance was determined by using χ^2 tests. In both sets of experiments we compared survivorship between hatching ages and predator types. We included all sequential 24 h experiments in one analysis and all sequential 72 h experiments in another analysis, acknowledging that this confounds effects of predator species with those of experiment date, which we expect to be small. In the 24 h experiments we also included age structure as a factor. Growth rates over 24 h (Experiment 1) and final tadpole lengths after 72 h (Experiment 2) were compared using an ANCOVA, with initial tadpole length as a covariate. The factors for the first experiment were hatching age, predator identity and hatchling age structure, and for the second experiment the effects of hatching age and predator identity were tested. In all analysis we started with a full factorial design and used stepwise model simplification based on the Akaike Information Criterion (AIC) to obtain the minimal adequate model (Online Appendix 1). All statistical tests were carried out in R software (R Development Core Team 2009). Confidence intervals and statistical contrasts between treatments were obtained using the “contrast” package (Kuhn et al. 2011).

Results

Experiment 1: Effects of hatchling phenotype and age structure on predation and growth rates

The best model to explain tadpole mortality during their first 24 h in the water included the effects of predator type ($\chi^2 = 86.59$, $df = 3$, $P < 0.0001$), hatching age ($\chi^2 = 21.55$, $df = 1$, $P < 0.0001$), and the interaction between these two factors ($\chi^2 = 37.04$, $df = 1$, $P < 0.0001$). This result indicates that consequences of early hatching depended upon predator species, but not hatchling age structure. Early-hatched tadpoles had on average 7.1 % (CI₉₅ 3.3–11.0 %) higher mortality than late hatchlings with belostomatids (Fig. 4a), and 16.3 % (CI₉₅ 10.8–21.9 %) lower mortality than late hatchlings with aeshnids (Fig. 4a). Hatching age did not affect libellulid predation over 24 h, which was low in both early and late-hatched treatments (Fig. 4a). In the absence of predators all late-hatched tadpoles survived and 3 % of the early hatchlings died within 24 h (Fig. 4a).

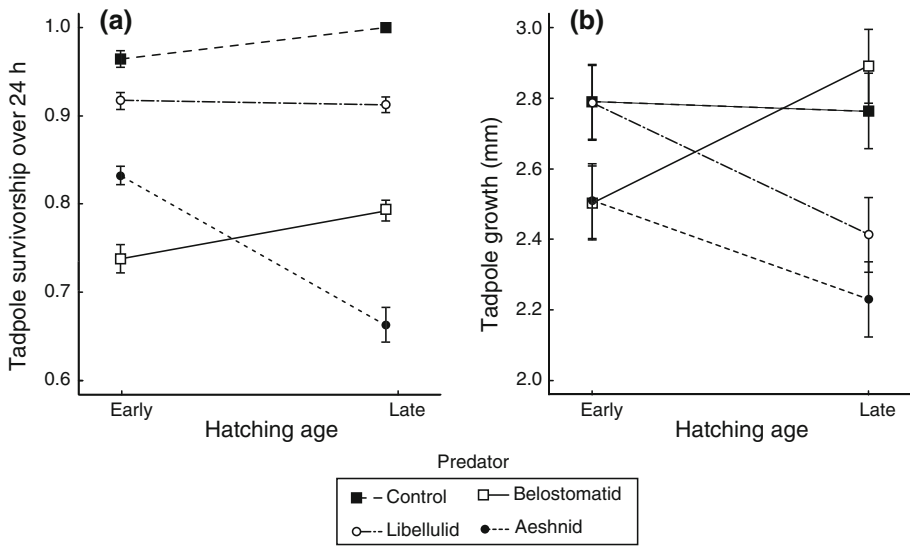


Fig. 4 Mean survivorship (a) and growth (b) of of early hatched and late-hatched tadpoles after 24 h of exposure to one of three insect predators or a predator-free control. Data are pooled across single and mixed age-structure treatments since age structure had no effect on survivorship or growth. Error bars show SE

Early hatchlings entered the water at 9.84 (SD = 0.44) mm, and late hatchlings at 12.00 (SD = 0.63) mm. Tadpole growth, as measured by total length, was best explained by a model including predator type, hatching age, the initial length covariate, and their three way interaction, but excluding age structure (Table 1). In most cases, smaller tadpoles grew faster within each hatching age class, but the effect of initial length on tadpoles growth differed among predatory environments and hatching age classes (Online Appendix 2). Once the effect of initial length was accounted for, growth was affected by the predator treatment and hatching age. Average-length early hatchlings and average-length late hatchlings had similar growth rates in predator-free environments (contrast = 0.03 mm, CI₉₅ -0.18 to 0.24 mm, Fig. 4b). However, where vulnerability differed between age classes, growth was faster for the less vulnerable age class (Fig. 4b). With belostomatids average-length late hatchlings grew 0.40 (CI₉₅ 0.19–0.61) mm more than average-length early hatchlings, and with aeshnid dragonflies they grew 0.28 (CI₉₅ 0.07–0.49) mm less. With libellulid dragonflies, which posed a relatively low and equal risk to both hatching age classes, early hatchlings grew 0.38 (CI₉₅ 0.17–0.59) faster than late hatchlings (Fig. 4b).

Experiment 2: Effects of hatching timing on survival through early ontogeny

Measured at age 7 days, when the effects of both hatchling phenotype and the partitioning of early development between safe egg and dangerous larval environments were combined, early hatched tadpoles had lower survivorship than late-hatched tadpoles with all three predators ($\chi^2 = 11.983$, $df = 1$, $P = 0.0005$, Fig. 5). However, mortality differed among predators ($\chi^2 = 72.912$, $df = 2$, $P < 0.0001$, Fig. 5). After 72 h, belostomatids and aeshnids had consumed over half of the early hatched tadpoles, while predation by libellulids remained relatively low (Fig. 5a). Even though two additional days of exposure to aquatic predators resulted in higher mortality of early versus late hatchlings with all

Table 1 Minimal adequate ANCOVA explaining tadpole growth during their first 24 h in the water

Source	<i>df</i>	MS	F	<i>P</i>
Predator	3	1.23	10.71	<0.0001
Hatching age	1	0.20	1.70	0.194
Initial length	1	18.83	163.04	<0.0001
Predator × Hatching age	3	0.40	3.50	0.017
Predator × Initial length	3	0.28	2.38	0.072
Hatching age × Initial length	1	0.25	216	0.144
Predator × Hatching age × Initial length	3	0.67	5.79	<0.001
Residuals	145	0.12		

Early and late hatched tadpoles were measured upon hatching and exposed to one of three predators (belostomatid, aeshnid or libellulid) or a no-predator control, in tanks with age structure (both age classes present) and tanks without it (a single age class per tank). Significant effects are highlighted in bold

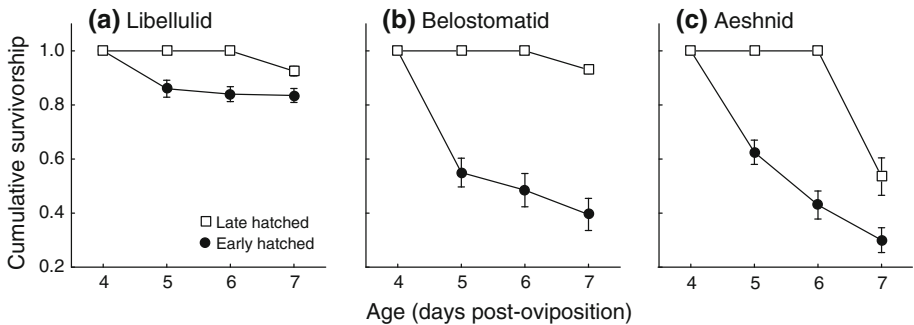


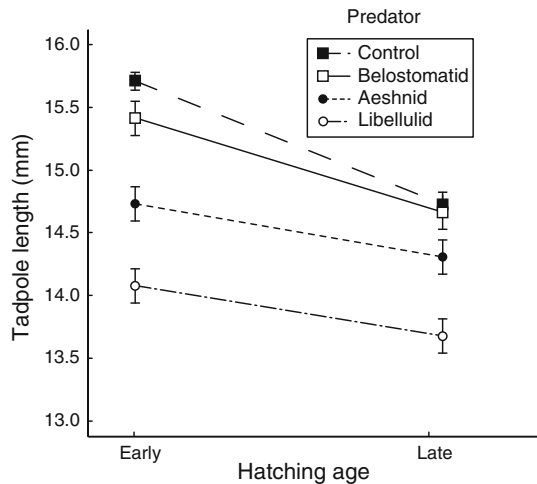
Fig. 5 Cumulative survivorship of early and late-hatched tadpoles during the 72 h period between the onset of hatching competence and 1 day after the peak of spontaneous hatching. After hatching, tadpoles were exposed to either: **a** libellulid dragonfly nymphs, **b** belostomatid giant water bugs, or **c** aeshnid dragonfly nymphs. Late-hatched tadpoles experienced no predation before hatching; they are shown with zero mortality during their late embryonic period, as is typical for undisturbed clutches. Data are mean and SE of $N = 10$ replicate tanks per treatment

predators, a significant interaction ($\chi^2 = 19.993$, $df = 2$, $P < 0.0001$, Fig. 5) shows that differences in survivorship with hatching age were larger with the belostomatid (53.5 %) than with either dragonfly (23.5 % with aeshnids and 9.0 % with libellulids). Tadpole length at the end of this experiment was affected by their hatching age ($F_{1,56} = 13.44$, $P = 0.005$), with early hatched tadpoles consistently larger than late-hatched tadpoles. Final size also varied across experiments with different predators ($F_{1,56} = 22.14$, $P < 0.001$; Fig. 6).

Discussion

This study aimed to understand the role of environmental context in shaping the fitness trade-off that maintains plasticity of hatching timing in the red-eyed treefrog. We assessed larval mortality costs of induced early hatching under two naturally variable elements of the larval environment, hatching age structure and predator identity. Also, we asked if predation risk is associated with reduced growth, even before tadpoles start feeding.

Fig. 6 Total length of early hatched and late-hatched tadpoles of the same egg cohort but different larval environments at 7 days of age. Hatchlings were exposed to one of three predatory insects. Early hatched tadpoles developed in the water for 72 h while late-hatched tadpoles developed in their eggs for two more days and in the water for only 24 h. Data are mean and SE of $N = 10$ replicate tanks per predator treatment. Final length of 12 predator-free controls (9 early and 3 late-hatched) is also presented



Previous studies indicate that hatching timing affects the behavior, morphology and growth of tadpoles (Warkentin 1999a, b; Vonesh and Bolker 2005), as well as the outcome of interactions with predators of larval and post-metamorphic stages (Warkentin 1995; Vonesh and Osenberg 2003; Vonesh 2005; Gomez-Mestre et al. 2008b; Touchon and Warkentin 2010). However, the magnitude and even the direction of these effects vary across studies, probably because consequences of early hatching differ when assessed in different predatory environments or at different points in ontogeny. We found that predator identity and foraging behavior influence and may reverse the immediate effects of hatchling phenotype on mortality rate, while modulating growth rates of hatchlings. Nonetheless, the hypothesis that a general predation risk trade-off exists across life stages is supported by the finding that, once the effects of accelerated hatching on the partitioning of early development across stages are factored in, mortality costs become apparent regardless of the predator to which larvae are exposed.

We found that the effect of hatching timing on initial larval mortality rate depended on the predator. Hatching early increased mortality with a large active forager (belostomatid), had a negligible effect with a small active forager (libellulid), and seemed advantageous with an ambush predator (aeshnid). Early hatchlings are not only smaller than spontaneously hatched tadpoles, but also less active (Online Appendix 3) and they have slower, shorter escape responses (Landberg et al. 2011) and a shorter flight initiation distance (Warkentin 1999a). Low activity and late, slow escape responses are disadvantageous with active foragers that pursue their prey (e.g. Feder 1983; Watkins 1996; Pruitt et al. 2012) and probably cause high mortality of early hatchlings with belostomatids, as they do with predatory shrimp (Warkentin 1999a). On the other hand, immobility protects prey against visually oriented sit-and-wait predators (Skelly 1994; Hatle et al. 2002), such as aeshnids. Higher predation by aeshnids on late than on early hatchlings is consistent with another study (Landberg et al. 2011) and with predictions from behavior. Mortality in control tanks was rare compared to predation but it occurred only to early hatchlings. This suggests that viability costs may exist for tadpoles hatched at the onset of hatching competence, even in the absence of predators. Indeed, a recent longer-term study also found that early hatching increases mortality in predator-free venues (Touchon et al. 2013). We found no evidence that age structure modulated risk, with any predator, for either hatchling age class. It is, however, still possible that the greater, more complex age structure found in ponds might

mitigate predation costs of early hatching, or that the presence of hatchlings might mitigate risk for some older tadpoles in nature.

Our results indicate that predators can suppress tadpole growth even in the first day after hatching, but that the strength of this effect varies with predator type and hatching age. Hatchling growth suppression by predators cannot be due to reduced foraging. All *A. callidryas* hatch with substantial yolk reserves and at 24 h post-hatching early hatchlings have never and late hatchlings rarely begun feeding (Warkentin 1999b). Instead, slower growth might be caused by stress in the presence of predators. Producing and maintaining the stress proteins that increase metabolism in preparation for the fight-or-flight response is costly, and this can be reflected in decreased growth rates (Slos and Stoks 2008). The differences across hatching ages in growth suppression with each predator were largely congruent with relative risk (Fig. 4a, b). In particular, with the two more voracious predators, each age class responded more strongly to the predator to which it was more vulnerable. Nonetheless, across predator environments we found that by age 7 day early hatched tadpoles were larger than late-hatched tadpoles from the same egg cohort. Thus the growth-suppressive effects of exposure to aquatic predators are not stronger than the enhanced growth rate associated with leaving the constraints of the egg capsule (Warkentin 1999b).

The phenotypic effects on predation discussed above are an incomplete picture of the short-term mortality costs of accelerated hatching because hatching early also extends the period of exposure to aquatic predators. We hypothesized that this shift in the partitioning of early development across life stages, and environments, may override any initial benefits of early hatchling phenotypes. Our results support this idea. Cumulative mortality revealed costs of early hatching even with a predator that caused relatively low and equal initial mortality rates (libellulids) and reversed the initial phenotypic advantage of early hatchlings with aeshnids. Phenotypic and time-partitioning effects differ drastically with aeshnids in part because for early hatchlings aquatic risk accumulates over more time and also because as early hatchlings develop and become more active they rapidly become more detectable to ambush predators. These short-term effects of time partitioning across life stages are important because differences in survivorship between hatching ages that are established in a brief period after hatching can persist through larval ontogeny to affect recruitment into the juvenile stage (Touchon et al. 2013).

In frog species studied so far, the onset of hatching competence and the timing of spontaneous hatching evolve independently (Gomez-Mestre et al. 2008b; Gomez-Mestre and Warkentin 2013). The lower limit of inducible hatching is probably set by the development of the hatching mechanism and has evolved under selection by acute egg-stage risk. Across *A. callidryas* and several other amphibians the onset of inducible hatching represents only 56–80 % of the developmental period before spontaneous hatching (Warkentin 2011b). The fact that safe embryos rarely hatch as early as they can suggests that costs of early hatching are common. Our results point to predation risk as a possible general mechanism for this cost, even with unpredictable variation in the post-hatching environment. Early hatchlings have more time to die during a period when they are highly vulnerable to some predators and increasingly vulnerable to others.

Acknowledgments We thank the Autoridad Nacional del Ambiente de Panamá for permission to conduct this research (Permits SE/A-16-10 and SC/A-23-12) and the Smithsonian Tropical Research Institute for use of their facilities and logistical support. Animal use was approved by STRI (protocol 100625-1008-15) and Boston University (protocol 08-011). We thank Z. Costa, R. Greene, R. Komosinski, C. Noss, S. Schleier, and J. Touchon for assistance. Comments by two anonymous reviewers, the associate editor and the editor John Endler greatly improved the manuscript. Funding was provided by the National Science Foundation,

USA (DEB-0716923 to K.M.W and 0717220 to J.R.V), Boston University, Virginia Commonwealth University and the Smithsonian Tropical Research Institute.

References

- Chrzanowski TH, Simek K (1990) Prey-size selection by freshwater flagellated protozoa. *Limnol Oceanogr* 35:1429–1436
- Cloarec A (1990) Factors influencing the choice of predatory tactics in a water bug, *Diplonychus indicus* Venk. & Rao (Heteroptera: Belostomatidae). *Anim Behav* 40:262–271
- de Roos AM, Persson L, Thieme HR (2003) Emergent Allee effects in top predators feeding on structured prey populations. *Proc R Soc B* 270:611–618
- Doody JS, Paull P (2013) Hitting the ground running: environmentally cued hatching in a lizard. *Copeia* 2013:159–164
- Feder ME (1983) The relation of air breathing and locomotion to predation on tadpoles, *Rana berlandieri*, by turtles. *Physiol Zool* 56:522–531
- Gomez-Mestre I, Warkentin KM (2007) To hatch and hatch not: similar selective trade-offs but different responses to egg predators in two closely related, syntopic treefrogs. *Oecologia* 153:197–206
- Gomez-Mestre I, Warkentin KM (2013) Risk-induced hatching timing shows low heritability and evolves independently of spontaneous hatching in red-eyed treefrogs. *J Evol Biol* 26:1079–1089
- Gomez-Mestre I, Touchon JC, Warkentin KM (2006) Amphibian embryo and parental defenses and a larval predator reduce mortality from water mold. *Ecology* 87:2570–2581
- Gomez-Mestre I, Touchon JC, Saccocio VL, Warkentin KM (2008a) Genetic variation in pathogen-induced early hatching of toad embryos. *J Evol Biol* 21:791–800
- Gomez-Mestre I, Wiens JJ, Warkentin KM (2008b) Evolution of adaptive plasticity: risk-sensitive hatching in neotropical leaf-breeding treefrogs. *Ecol Monogr* 78:205–224
- Grant JWA, Noakes DLG (1987) Movers and stayers: foraging tactics of young-of-the-year brook charr, *Salvelinus fontinalis*. *J Anim Ecol* 56:1001–1013
- Greene CH (1986) Patterns of prey selection: implications of predator foraging tactics. *Am Nat* 128:824–839
- Hatle JD, Salazar BA, Whitman DW (2002) Survival advantage of sluggish individuals in aggregations of aposematic prey, during encounters with ambush predators. *Evol Ecol* 16:415–431
- Huey RB, Pianka ER (1981) Ecological consequences of foraging mode. *Ecology* 62:991–999
- Ireland PH (1989) Larval survivorship in two populations of *Ambystoma maculatum*. *J Herpetol* 23:209–215
- Ireland DH, Wirsing AJ, Murray DL (2007) Phenotypically plastic responses of green frog embryos to conflicting predation risk. *Oecologia* 152:162–168
- Kuhn M, Weston S, Wing J, Forester J (2011) Contrast: a collection of contrast methods. R package version 0.17. <http://CRAN.R-project.org/package=contrast>
- Landberg T, Cohen KL, Willink B, Warkentin KM (2011) Effects of hatching age and predator cues on the development of escape swimming performance and survival with dragonfly predators in red-eyed treefrogs. *Integr Comp Biol* 51:E76
- Li D (2002) Hatching responses of subsocial spitting spiders to predation risk. *Proc R Soc B* 269:2155–2161
- McCoy MW, Bolker BM, Warkentin KM, Vonesh JR (2011) Predicting predation through prey ontogeny using size-dependent functional response models. *Am Nat* 177:752–766
- Michel MJ, Adams MM (2009) Differential effects of structural complexity on predator foraging behavior. *Behav Ecol* 20:313–317
- Moran NA (1992) The evolutionary maintenance of alternative phenotypes. *Am Nat* 139:971–989
- Moreira PL, Barata M (2005) Egg mortality and early embryo hatching caused by fungal infection of Iberian rock lizard (*Lacerta monticola*) clutches. *Herpetol J* 15:265–272
- Preisser EL, Orrock JL, Schmitz OJ (2007) Predator hunting mode and habitat domain alter nonconsumptive effects in predator–prey interactions. *Ecology* 88:2744–2751
- Pritchard G (1965) Prey capture by dragonfly larvae (Odonata; Anisoptera). *Can J Zool* 43:271–289
- Pruitt JN, Stachowicz JJ, Sih A (2012) Behavioral types of predator and prey jointly determine prey survival: potential implications for the maintenance of within-species behavioral variation. *Am Nat* 179:217–227
- Rogge JR, Warkentin KM (2008) External gills and adaptive embryo behavior facilitate synchronous development and hatching plasticity under respiratory constraint. *J Exp Biol* 211:3627–3635
- Rosenheim JA, Corbett A (2003) Omnivory and the indeterminacy of predator function: can a knowledge of foraging behavior help? *Ecology* 84:2538–2548
- Sherk TE (1977) Development of the compound eyes of dragonflies (Odonata). I. Larval compound eyes. *J Exp Zool* 201:391–416

- Sherratt TN, Harvey IF (1989) Predation by larvae of *Pantala flavescens* (Odonata) on tadpoles of *Phyllomedusa trinitatis* and *Physalaemus pustulosus*: the influence of absolute and relative density of prey on predator choice. *Oikos* 56:170–176
- Skelly DK (1994) Activity level and the susceptibility of anuran larvae to predation. *Anim Behav* 47:465–468
- Slos S, Stoks R (2008) Predation risk induces stress proteins and reduces antioxidant defense. *Funct Ecol* 22:637–642
- Tanhuanpää M, Ruohomäki K, Uusipaikka E (2001) High larval predation rate in non-outbreaking populations of a geometrid moth. *Ecology* 82:281–289
- Touchon JC, Warkentin KM (2010) Short- and long-term effects of the abiotic egg environment on viability, development and vulnerability to predators of a Neotropical anuran. *Funct Ecol* 24:566–575
- Touchon JC, Gomez-Mestre I, Warkentin KM (2006) Hatching plasticity in two temperate anurans: responses to a pathogen and predation cues. *Can J Zool* 84:556–563
- Touchon JC, McCoy MW, Vonesh JR et al (2013) Effects of hatching plasticity carry over through metamorphosis in red-eyed treefrogs. *Ecology* 94:850–860
- Vonesh JR (2005) Sequential predator effects across three life stages of the African tree frog, *Hyperolius spinigularis*. *Oecologia* 143:280–290
- Vonesh JR, Bolker BM (2005) Compensatory larval responses shift trade-offs associated with predator-induced hatching plasticity. *Ecology* 86:1580–1591
- Vonesh JR, Osenberg WW (2003) Multi-predator effects across life-history stages: non-additivity of egg- and larval-stage predation in an African treefrog. *Ecol Lett* 6:503–508
- Warkentin KM (1995) Adaptive plasticity in hatching age: a response to predation risk trade-offs. *Proc Natl Acad Sci USA* 92:3507–3510
- Warkentin KM (1999a) The development of behavioral defenses: a mechanistic analysis of vulnerability in red-eyed tree frog hatchlings. *Behav Ecol* 10:251–262
- Warkentin KM (1999b) Effects of hatching age on development and hatchling morphology in the red-eyed treefrog, *Agalychnis callidryas*. *Biol J Linn Soc* 68:443–470
- Warkentin KM (2000) Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. *Anim Behav* 60:503–510
- Warkentin KM (2002) Hatching timing, oxygen availability, and external gill regression in the tree frog, *Agalychnis callidryas*. *Physiol Biochem Zool* 75:155–164
- Warkentin KM (2011a) Environmentally cued hatching across taxa: embryos respond to risk and opportunity. *Integr Comp Biol* 51:14–25
- Warkentin KM (2011b) Plasticity of hatching in amphibians: evolution, trade-offs, cues and mechanisms. *Integr Comp Biol* 51:111–127
- Warkentin KM, Currie CC, Rehner SA (2001) Egg-killing fungus induces early hatching of red-eyed treefrog eggs. *Ecology* 82:2860–2869
- Warkentin KM, Buckley CR, Metcalf KA (2006) Development of red-eyed treefrog eggs affects efficiency and choices of egg-foraging wasps. *Anim Behav* 71:417–425
- Watkins TB (1996) Predator-mediated selection on burst swimming performance in tadpoles of the Pacific Tree Frog, *Pseudacris regilla*. *Physiol Zool* 69:154–167
- Wedekind C (2002) Induced hatching to avoid infectious egg disease in whitefish. *Curr Biol* 12:69–71
- Werner EE (1986) Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *Am Nat* 128:319–341
- Werner EE, Gilliam JF (1984) The ontogenetic niche shift and species interactions in size-structured populations. *Annu Rev Ecol Evol* 15:393–425