

Consequences of induced hatching plasticity depend on predator community

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Abstract Many prey species face trade-offs in the timing of life history switch points like hatching and metamorphosis. Costs associated with transitioning early depend on the biotic and abiotic conditions found in the subsequent life stage. The red-eyed treefrog, *Agalychnis callidryas*, faces risks from predators in multiple, successive life stages, and can hatch early in response to mortality threats at the egg stage. Here we tested how the consequences of life history plasticity, specifically early hatching in response to terrestrial egg predators, depend on the assemblage of aquatic larval predators. We predicted that diverse predator assemblages would impose lower total predation pressure than the most effective single predator species and might thereby reduce the costs of hatching early. We then conducted a mesocosm experiment where we crossed hatching

phenotype (early vs. normal hatching) with five larval-predator environments (no predators, either waterbugs, dragonflies, or mosquitofish singly, or all three predator species together). The consequences of hatching early varied across predator treatments, and tended to disappear through time in some predation treatments, notably the waterbug and diverse predator assemblages. We demonstrate that the fitness costs of life history plasticity in an early life stage depend critically on the predator community composition in the next stage.

Keywords Predator diversity · Hatching · Complex life cycles · *Agalychnis callidryas* · Life history plasticity

Introduction

Organisms with complex life cycles often exhibit plasticity in the timing of life history switch points, whether the switch point is colony sexual maturation in eusocial insects (Holland et al. 2013), anadromy or other ontogenetic niche shifts in fish (Werner and Gilliam 1984; Werner 1986; Aubin-Horth et al. 2009), or metamorphosis in amphibians and insects. Metamorphosis, in particular, has been studied across many taxa for nearly a century (Pruthi 1925; Adolph 1931), and a wide variety of organisms demonstrate flexible size at, and timing of, metamorphosis (Werner and Gilliam 1984; Werner 1986; Hentschel and Emlet 2000; Peckarsky et al. 2001; Benard 2004; Touchon et al. 2013). Some of this plasticity in metamorphosis is adaptive, for example, as a response to larval predation risk or pond drying (Newman 1992), whereas some may be the non-adaptive consequence of variation in environmental conditions during the larval period (Hentschel and Emlet 2000; Juliano et al. 2004; Touchon et al. 2013).

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In contrast to metamorphosis, plasticity in the timing of hatching from the egg is much less well appreciated (Warkentin 1995; Miner et al. 2010; Christy 2011; Martin et al. 2011). Several recent reviews document hatching plasticity in species throughout the Bilateria, in response to cues that range from physical disturbance to natural enemies and resource limitation (Doody 2011; Warkentin 2011a, 2011b). Thus, induced changes in life history switch points are common, and the phenotypic differences arising from them may have important consequences for shaping species interactions and performance in subsequent stages (Altwegg and Reyer 2003; Vonesh and Bolker 2005; Orizaola et al. 2010; Dahl et al. 2012; Touchon et al. 2013).

Predators in aquatic communities have long been known to alter the abundance, distribution, morphology, and behavior of their prey (Sih et al. 1985). However, predators can also influence the timing of life history switch points and habitat transitions of their prey (Werner 1986; Peckarsky et al. 2001; Benard 2004; Smith and Fortune 2009). Hatching early in response to egg predators has the clear advantage of removing eggs from that risky environment, but may leave the newly hatched larvae smaller and poorly developed (Warkentin 1995; Chivers et al. 2001; Li 2002; Kusch and Chivers 2004; Warkentin 2011b; Touchon et al. 2011), less capable of locomotion (Warkentin 1999; Buckley et al. 2005), and thus change their susceptibility to predators in the larval environment (Vonesh and Bolker 2005). Being smaller or less motile may increase or decrease predation risk in the short term, depending on size selectivity and foraging mode (e.g., sit-and-wait vs. active) of the larval predators. However, entering the larval environment smaller may increase the duration of the larval period, and thus increase the cumulative predation risk across an individual's lifetime (Willink et al. 2013), unless the early hatched larvae can compensate via faster growth and/or developmental rates (Vonesh and Bolker 2005; Touchon and Warkentin 2010).

How predator diversity per se, or particular combinations of predator species' traits in a diverse assemblage, might influence prey communities is of great interest (e.g., Soluk 1993; Sih et al. 1998; Finke and Denno 2004). Emergent multiple-predator effects, arising from differences in predator traits or from direct or indirect interactions among predator species, can cause the combined effects of predators on a shared prey to deviate from predictions based on their independent effects (Sih et al. 1998; Relyea 2003). For example, predator species with complementary habitat domains may chase prey into each other's domains, enhancing risk to the prey [i.e., remove potential refugia (Schmitz 2007)]. Alternatively, intraguild predation may reduce risk to prey by reducing predator density or altering predator behavior (Sih et al. 1998). Thus, the viability of different prey phenotypes (i.e., early or late-

prey) should depend on predator diversity and composition. However, few if any studies of the consequences of egg-hatching plasticity have considered prey that face multiple larval predator species simultaneously, which of course is the most likely state in natural ecosystems.

Here we examine how the consequences of plastic responses to predators in an early terrestrial life stage depend on predator community diversity and composition in the subsequent aquatic life stage. Our focal prey species is the leaf-breeding Neotropical red-eyed treefrog, *Agalychnis callidryas*. The arboreal eggs of this species hatch early in response to attacks by a suite of egg predators and pathogens (Warkentin 1995, 2000). However, hatching early to escape egg predators can be costly, reducing subsequent larval viability (Touchon et al. 2013) and altering interactions with aquatic predators. In predation trials with single predator species, early hatched tadpoles are more vulnerable than late-hatched tadpoles to fish, shrimp, backswimmers, and waterbugs, similarly vulnerable to libellulid dragonflies, and less vulnerable to aeshnid dragonflies (Warkentin 1995; Willink et al. 2013). Unknown, however, is how these larval predators may interact in diverse assemblages, and how these predator communities may in turn affect trade-offs associated with flexible hatching timing.

Materials and methods

Study system

This study was conducted at the Smithsonian Tropical Research Institute field station in Gamboa, Panama (9°7'0N and 79°42'0W). In a survey of five local ponds, *A. callidryas* ranged in density from 0 to 793 individuals/m², and ponds contained ~ ten species of tadpole predators including multiple fish, odonate, and waterbug species, as well as fishing spiders, shrimp, diving beetle larvae and backswimmers. All ponds had more than four distinct functional/taxonomic groups of predators present (J. C. Touchon and J. R. Vonesh, unpublished data). We focused on three naturally co-occurring predators that differ in functional morphology and foraging mode: a dragonfly naiad (*Pantala flavescens*, Odonata: Libellulidae), a mosquitofish (*Gambusia nicaraguensis*, Cyprinodontiformes: Poeciliidae), and a relatively small species of waterbug (*Belostoma* sp., Hemiptera: Belostomatidae). Odonate larvae and waterbugs co-occur in all ponds in the Gamboa area and fish are found in approximately half of ponds (J. C. Touchon and J. R. Vonesh, unpublished data). *Pantala flavescens* is typically a sit-and-wait predator, with a benthic habitat domain, but will swim through the water column to attack tadpoles (Corbet 1999). Waterbugs breathe air at the surface and often perch there waiting for prey, but have good visual

perception and can easily swim throughout the water column. Both larval dragonflies and waterbugs are known to cannibalize conspecifics (McPeck and Crowley 1987; Van Buskirk 1992; Smith 1974; Ohba et al. 2006). Mosquitofish are actively swimming, visual predators that readily prey upon amphibian larvae (Komak and Crossland 2000; Baber and Babbitt 2004), including *A. callidryas* tadpoles (Warkentin 1995). Mosquitofish, being gape-limited and not much larger than larval amphibians, often wound or maim individual tadpoles, killing them through multiple injuries rather than consuming them whole in a single attack. Thus, these predator species represent a range of foraging modes and have only partially overlapping habitat domains.

Predator community mesocosm experiment

We conducted a randomized, complete block, factorial experiment crossing early or late-hatching tadpoles (4 or 6 d.p.o., respectively) with five predator diversity and identity treatments, using 400-L outdoor mesocosms (“tanks”, 0.7-m-diameter base, 0.9-m-diameter mouth, and 0.8-m-high, with screened drain holes at 0.75-m height) as experimental units. The five predator treatments were: no-predator control treatments, single-predator treatments containing three individuals of one predator species (waterbugs, dragonflies, or mosquitofish), and a mixed-predator treatment containing one individual of each of the three predator species. Replacement series (or “substitutive”) designs such as this avoid the confounding changes in total predator density that complicate the analysis of additive designs (Schmitz 2007).

The experiment consequently had ten unique hatching/predator treatments, which were replicated six times, once in each of six blocks for a total of 60 tanks. Simultaneous set-up of the entire experiment was precluded by the number of *Agalychnis* egg masses that could be found on any particular day; thus two complete blocks were initiated on three separate dates (16 August, 3 September, 23 September 2009). The “block” factor therefore subsumes variation in responses attributable to spatial and temporal differences.

Tanks were first filled with a mixture of aged tap water and collected rain water. The following day a diverse collection (20 L for each set of two blocks) of zooplankton, phytoplankton, and periphyton was obtained from nearby ponds, sieved to remove larger invertebrates, and 1 L was added to each tank. A commercial fish/tadpole food (Sera Micron powdered food; 7.5 g per tank) was added the next day to supply nutrients to the newly established communities. Tanks were covered with fiberglass window screening to prevent entry/exit of organisms.

On the third day following filling, 20 *Physa* sp. snails (6.9 ± 0.8 mm shell height) were added to each tank. Leaf

litter was collected from nearby trees, bleached overnight, triple rinsed, air dried, then weighed at ambient moisture levels. To provide substrate for microbes, 200 g of this leaf litter was added to a window screen mesh enclosure and placed in each tank five days after filling. The bag contained the litter so subsequent tank checks for tadpole growth and survival would be feasible. An additional 50 g of leaf litter was added on the sixth day post-filling to act as benthic substrate and refuge for the tadpoles. The addition of plankton, snails, and leaf litter added realism to the experiment, but also allowed us to study the indirect effects of the focal predator–prey interactions on the larger communities (reported elsewhere, unpublished manuscript). Tanks, covers, and any items used to sample tanks were also bleached and rinsed before and after use to reduce the possibility of disease.

Agalychnis eggs were collected from two ponds—Experimental Pond (a concrete pond at the forest edge, filled with rain water and colonized by natural flora and fauna) and Ocelot Pond (a larger, forested pond ~2.5 km from the tank array), and raised in the lab. To obtain early and late-hatching tadpoles on the same day, sets of between seven and 13 clutches were collected from Experimental and Ocelot ponds on each of two mornings 48 h apart. Late-hatching tadpoles (10.8 ± 1.3 mm total length) were allowed to develop normally, hatching six days after being laid. Early hatching tadpoles (9.0 ± 0.9 mm total length), from clutches laid two days later, were induced to hatch by mechanical agitation and hypoxia from submersion in water. Some lingering late-hatching embryos were also induced to hatch by brief physical contact. Hatchlings from different clutches of the same hatching age were mixed and groups of 50 *Agalychnis* tadpoles were haphazardly selected and added to each tank, just after hatching.

Predators were collected from local aquatic habitats. Several days before addition to the experiment, fish were fed newly hatched tadpoles because previous observations indicated that while capable of depredating tadpoles, the fish often needed prior experience before consuming them readily (K. Warkentin, personal observation). All predators were then starved for 48 h before the experiment started. Before the experiment began, all animals were placed in shallow trays of water, and dorsal views were photographed with a Nikon D70s digital camera. Total lengths were then measured with ImageJ version 1.43 image analysis software (Rasband 1997). Predators in the mesocosm experiment had average lengths of 13.0 ± 1.3 mm for larval dragonflies, 12.9 ± 2.2 mm for waterbugs, and 28.2 ± 3.0 mm for fish. Dragonfly predators that metamorphosed to adults during the experiment were replaced with larval dragonflies. Other predators found dead were not replaced, as intraguild predation was of interest in the study.

We measured tadpole growth and survival weekly for 4 weeks by capturing every tadpole, in each tank, with dipnets. We continued netting each tank until ten consecutive dipnets produced no tadpoles. Total tadpole lengths were measured by photography and image analysis as described above. Predators were readily caught with this netting procedure, so the number of each still alive was likewise recorded.

We only consider data from the first and last time sampling periods (i.e., at 7 and 28 days after hatching) here because we were interested primarily in the initial costs of the early hatching phenotype during the likely period of greatest predation risk (first week) and the longer term costs that might accrue leading up to metamorphosis. Figures that match those presented here, but that include data from day 14 and 21, are in online appendices (Figs. A1-A3 in Supplementary material).

The proportional survival of tadpoles and predators were each modeled with generalized linear mixed models (GLMMs) with binomial error distributions [using function `glmer` in package `lme4` (Bates and Maechler 2009)]. Predator treatments, hatching treatments, and time (i.e., sampling day) and their interactions were modeled as fixed effects. Time was treated as a discrete factor. We used random slopes and intercepts models, with block, and tank nested within block, as random intercepts to account for correlations between individuals from the same block or tank respectively, and time as a random slope to account for repeated sampling of tanks (Zuur et al. 2009). In the event of a significant three-way interaction between hatching age, predator treatment and time, we conducted follow-up analyses testing for effects of hatching age and time, and their interaction, separately for each predator treatment. Two measurements of tadpole mortality, one each from the waterbug and dragonfly treatments and both on day 28, were outliers (i.e., very high mortality seemingly not due to treatment) and were excluded from analyses. Our data exploration (i.e., outlier checks) and model validation (i.e., visualizing residuals vs. predicted values and covariates, plots of predictions vs. observed data) supported the modeling approach outlined above, and revealed very little overdispersion in these models. Tadpole lengths were analyzed with a linear mixed model with a Gaussian error distribution, with a model structure and validation procedure otherwise similar to the analysis of tadpole survival described above. Survival rates of individual predator species in the all-predator treatment would have been compared with a binomial GLMM as above for overall predator survival, except there was no variation in survival among replicates in several instances (i.e., 0 or 100 % survival) which precluded this type of analysis.

Predictions

The three predator species seemed likely to play unique roles in the food web, and intraguild predation was expected to be strong in some cases. From small-scale pilot studies, personal experience, and knowledge of the system and species, we made the following predictions:

1. Waterbugs would kill other predators and cannibalize each other (Ohba et al. 2006) and thus the diverse predator treatment would be much like the waterbug treatment, with both resulting in a single waterbug preying on tadpoles.
2. The fish-only treatment would have the lowest tadpole survival, since it had voracious predators that were unlikely to cannibalize each other (Warkentin 1995).
3. Early hatching tadpoles would suffer greater mortality in general (Warkentin 1995; Willink et al. 2013).
4. The majority of tadpole mortality would occur by seven days post-hatching and mortality would decrease through time as surviving tadpoles grew in size and became less vulnerable to predators (Warkentin 1995; Touchon et al. 2013).

Results

Predator survival

Across all treatments, total predator density decreased from three individuals initially to a mean of 1.2 ± 0.2 (± 1 SE) predator individuals by the end of the experiment (time effect; Table 1; Fig. 1a; Fig. A1 in Supplementary material). However, the intensity and timing of mortality differed among predator treatments (significant predator \times time interaction; Table 1; Fig. 1a): fish survived well throughout the experiment, waterbugs decreased rapidly in abundance by day seven, and dragonfly survival was lowest overall.

Within the all-predator treatment, dragonfly larvae were extirpated by day 28, while 58 and 67 % of waterbugs and fish remained alive, respectively. There appeared to be no major differences in predator survival across early or late-hatching treatments (Fig. 1b). Survival of all three predators decreased over time, but this was relatively minor for fish and waterbugs compared to dragonfly larvae.

Comparisons of the survival of predators through the end of the experiment with conspecifics (Fig. 1a) or other species (Fig. 1b) suggest: dragonfly larvae had universally low survival (12 and 0 % with conspecifics and other species, respectively), waterbugs with conspecifics had lower survival (36 %) than when with other species (58.3 %), most likely due to cannibalism, and fish survived well in all

Table 1 Summary of statistical results from the predator diversity mesocosm experiment

Predator survival		Binomial GLMM		
Source	χ^2	<i>df</i>	<i>p</i>	
Predators (pred.)	20.70	3		0.0001
Hatching (hatch.)	0.419	1		0.517
Time	22.40	1		<0.0001
Pred. \times hatch.	3.55	3		0.315
Pred. \times time	11.02	3		0.012
Hatch. \times time	1.06	1		0.303
Pred. \times hatch. \times time	1.49	3		0.684
Tadpole survival		Binomial GLMM		
Source	χ^2	<i>df</i>	<i>p</i>	
Predators	40.41	4		<0.0001
Hatching	8.59	1		0.0034
Time	7.88	1		0.0050
Pred. \times hatch.	3.02	4		0.554
Pred. \times time	4.30	4		0.367
Hatch. \times time	2.82	1		0.093
Pred. \times hatch. \times time	12.75	4		0.013
Tadpole length		Gaussian LMM		
Source	χ^2	<i>df</i>	<i>p</i>	
Predators	21.57	4		<0.0001
Hatching	1.29	1		0.256
Time	24.04	1		<0.0001
Pred. \times hatch.	5.40	4		0.248
Pred. \times time	6.55	4		0.161
Hatch. \times time	0.20	1		0.652
Pred. \times hatch. \times time	5.77	4		0.217

GLMM Generalized linear mixed model

conditions (69.4 and 66.7 % in single-predator and diverse predator treatments, respectively).

Responses of *Agalychnis*

The effects of variable hatching time on tadpole survival through time depended on predator treatment (Fig. 2a–e; Fig. A2 in Supplementary material; Table 1; significant predator \times hatching \times time interaction). In essence, tadpoles that hatched two days early tended to suffer greater mortality than did late-hatching tadpoles (Fig. 2a–e), but this effect depended on the predator environment. Additionally, tadpole survival decreased rapidly in the first seven days of the experiment, but was followed by a reduction in mortality indicating that, as predicted, tadpoles were most vulnerable shortly after hatching (Fig. 2a; Table 1; significant time effect). In the predator-free tanks, there was no initial difference in tadpole survival among early and late-hatched tadpoles, but survival decreased over time and there was a trend for early hatched tadpoles to have

lower survival by the end of the experiment (Fig. 2a; no predators, hatching $\chi^2 = 0.84$, $P = 0.36$; time $\chi^2 = 4.85$, $P = 0.028$; hatching \times time $\chi^2 = 3.14$, $P = 0.076$). Early hatched tadpoles were initially more vulnerable to dragonfly larvae than were late-hatched tadpoles, and this pattern persisted until the end of the experiment (Fig. 2b; dragonfly predators, hatching $\chi^2 = 7.42$, $P = 0.006$; time $\chi^2 = 4.08$, $P = 0.043$; hatching \times time $\chi^2 = 1.56$, $P = 0.21$). There was a significant interaction between hatching age and time when *A. callidryas* tadpoles were with waterbug predators; early hatched tadpoles were initially more vulnerable to waterbugs, but survival of late-hatched tadpoles decreased over time such that after 28 days tadpole survival was equal for both hatching ages (Fig. 2c; waterbug predators, hatching $\chi^2 = 2.22$, $P = 0.14$; time $\chi^2 = 6.77$, $P = 0.009$; hatching \times time $\chi^2 = 4.66$, $P = 0.031$). Tadpole survival was by far the lowest in fish-only treatments and did not vary with hatching age (Fig. 2d; fish predators, hatching $\chi^2 = 2.38$, $P = 0.12$; time $\chi^2 = 6.83$, $P = 0.009$; hatching \times time $\chi^2 = 1.75$, $P = 0.19$). Lastly, similar to the waterbug-only

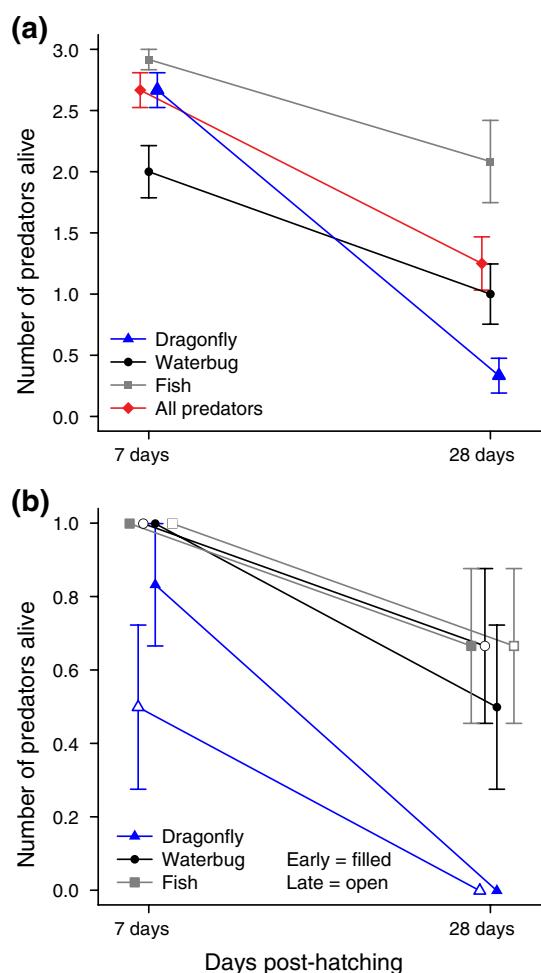


Fig. 1 **a** Average number of predators surviving in each predator treatment during the 28-day experiment, out of three total. **b** Average number of each type of predator surviving in the diverse predator treatment, shown for both early and late-hatching tadpole treatments. Means \pm 1 SE are presented

treatment, there was an interaction between hatching age and time in the diverse predator treatment such that early hatched tadpoles were more vulnerable after seven days,

but survival of all tadpoles was similar after 28 days (Fig. 2e; diverse predators, hatching $\chi^2 = 0.36$, $P = 0.55$; time $\chi^2 = 3.04$, $P = 0.08$; hatching \times time $\chi^2 = 8.46$, $P = 0.003$).

Although late-hatching tadpoles began the experiment larger than early hatching tadpoles, that initial difference disappeared quickly (i.e., by day seven), and there were no detectable size differences due to hatching timing later in the experiment (Fig. 3; Fig. A3 in Supplementary material; Table 1; non-significant hatch effect). Predator treatments did affect the size of tadpoles, with the smallest tadpoles being found in tanks with fish (Fig. 3; Table 1; significant predator effect). However, this effect on tadpole length most likely resulted from tail damage during predation attempts by fish (J. C. Touchon and J. M. Wojdak, in review). Tadpoles grew during the course of the experiment (Table 1; significant time effect), but there were no interactions between predator or hatching age treatments and the progression of growth through time.

Discussion

Hatching plasticity and the consequences for larvae

Phenotypic plasticity in the timing of life history transitions is common in a wide variety of taxa, enabling organisms to balance the benefits and costs associated with different life stages. Although presumably adaptive in the short term (e.g., escaping imminent predation risk), rushing through a developmental milestone may incur lasting costs and leave the organism less prepared for the next life stage (e.g., due to altered body size, energy reserves, motile speed, sensory perception, etc.). Red-eyed treefrogs that hatch ~30 % prematurely to escape egg predators are smaller, less developed, and more vulnerable to most predators (e.g., Warkentin 1995, 1999; Willink et al. 2013). Here, we demonstrated that the lasting consequences of that plastic life history shift

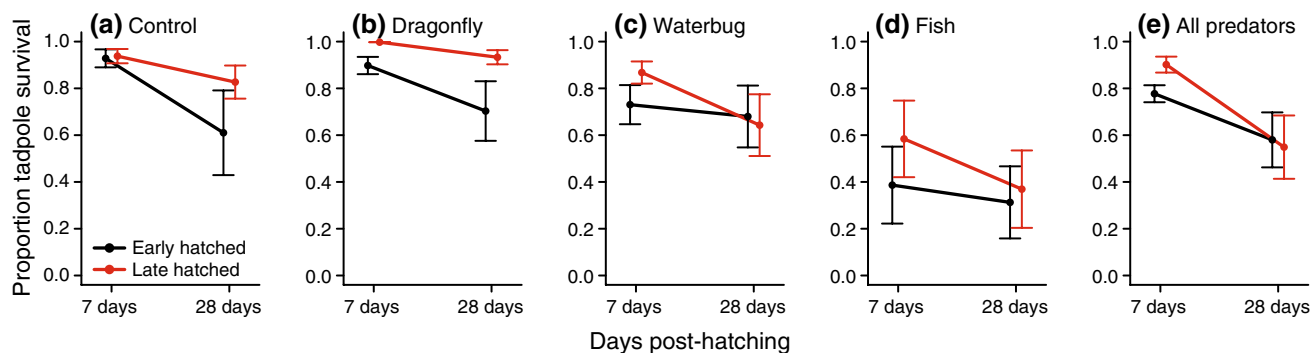


Fig. 2 Proportion of tadpoles surviving in the five predation treatments through the course of the 28-day experiment. Means \pm 1 SE are presented

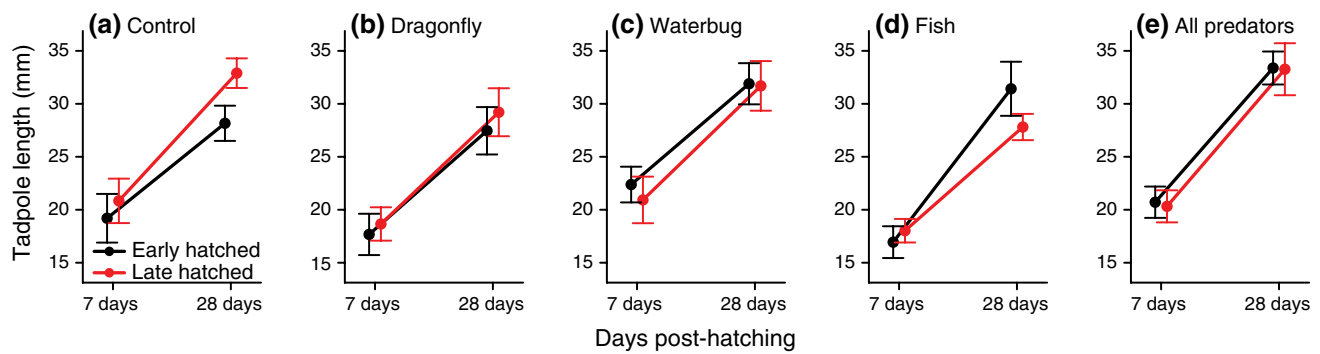


Fig. 3 Total length of tadpoles (average of mean length in millimeters per tank) in each predator treatment during the 28-day experiment. Means \pm 1 SE are presented

depended on the composition of the predator assemblage in the larval environment. Importantly, we demonstrated that latent effects of induced early hatching, at least in terms of survival and growth, were erased in a larval environment with a diverse predator community, the most realistic condition. Given the breadth of organisms that demonstrate flexible hatching timing [e.g., salamanders (Sih and Moore 1993); anurans (Touchon et al. 2006); crustaceans (Blaustein 1997); gastropods (Miner et al. 2010)], and the fact that few environments contain just one predator species, our results are likely widely applicable.

We detected significant short-term effects of hatching early on larval *A. callidryas* survival in three of our four predator environments (Fig. 2). Only when hatchlings entered an environment containing fish alone did we observe no initial significant effect of early hatching on tadpole survival, though the trend was for higher mortality among earlier hatched tadpoles (as predicted). We also detected a trend where survival of early hatched tadpoles was approximately 20 % lower than for late-hatched tadpoles after 4 weeks, in the absence of predators and in the presence of dragonflies (Fig. 2a, b). Touchon et al. (2013) found similar lasting effects of plastic hatching timing on red-eyed treefrog survival to metamorphosis, across variation in resource levels and the presence and absence of a predator (*P. flavescens*, the same dragonfly species we used here).

As predicted, the diverse predator treatment was most similar to the waterbug-only treatment with regards to tadpole survival (Figs. 2c, e), even though waterbugs did not prey upon fish (i.e., intraguild predation) to the degree that we predicted (Fig. 1b). There are at least two possible, and potentially overlapping explanations for this pattern. Firstly, if mosquitofish are highly effective predators only when in groups (e.g., “group attackers”), a single fish by itself may have little impact on overall mortality of tadpoles. Many predators, including some insects, carnivores, cetaceans, teleost fish, and arachnids are much

less successful hunters as individuals compared to when feeding in groups, particularly when the prey are relatively large compared to the predators (Caraco and Wolf 1975; Griffiths 1980). Secondly, the waterbugs may have altered mosquitofish behavior such that they were a less effective tadpole predator. A trait-mediated indirect interaction between waterbugs and tadpoles, mediated through fish, seems plausible, though we have no direct observational evidence of behavioral changes. Finke and Denno (2005) suggested that a similar mechanism contributed to the reduction in the strength of trophic cascades in salt marsh food webs when intraguild predators were present in diverse predator assemblages.

In the diverse predator treatment, dragonflies were eliminated by day 28, though most were present through the tadpole’s critical first week post-hatching. The initial presence and eventual extirpation of dragonflies in the diverse predation treatment likely had little effect—even when present as the sole predator, dragonflies had little impact on the tadpoles (comparing Fig 2a, b). Willink et al. 2013 similarly found *P. flavescens* dragonflies to be weak predators of *A. callidryas* tadpoles.

In both environments where waterbugs were present, we found that survival was initially lower for early hatched tadpoles, a result which is in accordance with other tadpole predators that are relatively small in body size, such as libellulid dragonfly naiads or backswimmers [Warkentin and Caldwell (2009); Touchon et al. (2013); but see Willink et al. (2013) for an example of equal mortality among early/late *A. callidryas* hatchlings in the presence of libellulids]. However, after 28 days survival was equal for both early and late-hatched individuals facing waterbug predation. Why was the cost of hatching early erased by waterbugs but not by dragonflies? Although it is difficult to know for certain, it is likely due to a combination of vulnerability of early versus late-hatched tadpoles, differences in the prey size limitation of each predator, and density-dependent encounter rates. Both predators consumed more early

hatched tadpoles during the first seven days of the experiment. Once waterbugs had reduced the density of early hatched individuals by 20–30 %, the remaining prey may have been at low enough density to have low encounter rates with waterbugs and therefore a low risk of predation. Since late-hatched tadpoles had greater survival during the first week, prey density remained high enough that predation continued over the course of the subsequent weeks. In contrast to the waterbugs, *P. flavescens* dragonfly naiads become gape-limited very quickly (McCoy et al. 2011). Differences in mortality evident after seven days, which resulted from the increased vulnerability of early hatched tadpoles, therefore persisted. In fact, the presence of dragonflies resulted in tadpole survival closer to the no predator controls than to any of the other predator treatments. Interestingly, Willink et al. (2013) did not detect a difference in survival between early and late-hatched tadpoles facing predation by *P. flavescens*. Their result may simply be a product of a single predator being quickly satiated by one or two individual prey (regardless of hatching treatment) over such a short time frame.

Prey size and hatching timing

Fish killed ~50 % of tadpoles by day seven, but very few after that point. This suggests that mosquitofish were efficient but gape-limited predators, only able to consume tadpoles when they were very small. Small-scale predation trials with fish and both small and medium-sized *Agalychnis* tadpoles support this conclusion (J. M. Wojdak and J. C. Touchon, unpublished data). We would have predicted that the costs of hatching early would be exacerbated for prey facing gape-limited predators, since early hatched individuals enter the larval environment smaller, and therefore are more vulnerable, potentially for a longer period of time. However, it may be that costs of hatching early are too subtle to be detected when prey are subjected to such strong predation risk. Alternatively, differences in mortality between early and late-hatched tadpoles may be obscured if early hatched individuals catch up in size quickly.

Indeed, early hatched tadpoles seem to have exhibited compensatory growth (sensu Hector and Nakawaga 2012) to overcome their initial size disadvantage. At hatching, when tadpoles were added to mesocosms to begin the experiment, late-hatched tadpoles were 16 % longer than early hatched tadpoles (see “Materials and methods”). By seven days post-hatching there were no clear differences between the sizes of the hatchling groups (Fig. 3). Interestingly, in the absence of predators there was a trend towards a renewed difference between late- and early hatched tadpoles in size (Fig. 3), although the three-way interaction between hatching, predator treatments, and time was not significant. Urban (2007) suggests that, in general, prey

facing gape-limited predators that themselves are growing (with increasing gape), might see fitness benefits from a fast-growing strategy “when size-based fecundity is high relative to gape-unconstrained predation risk and when gape-limited predation risk decelerates at higher prey growth rates”. More simply, when there is a gape-limited predator, there is a new benefit for *fast* growth—growth that does not outpace the predator carries all the risks associated with more foraging without the promise of reaching a size refuge.

Vonesh and Bolker (2005) found similar larval compensatory growth responses after induced early hatching in the East African reed frog, *Hyperolius spinigularis*. In that study, the initial compensation by early hatched larvae was so strong that they survived *better* than did later hatched larvae, mostly by growing through vulnerable size classes more rapidly. Costs of early hatching to *H. spinigularis* appeared late in the larval period and after metamorphosis, when individuals that had hatched early were smaller and thus less likely to survive adult predators (Vonesh and Bolker 2005).

Two mechanisms related to predation may influence the fitness consequences of life history plasticity—changes in vulnerability to predators and changes in the duration of the larval period (Werner 1986; Rowe and Ludwig 1991; van Uitregt et al. 2012). Even if our experiment had run to metamorphosis, the duration of the larval period for both early and late-hatched tadpoles would likely be the same—Warkentin (1999) and Touchon et al. (2013) both found no differences in time to metamorphosis for early or late-hatched *A. callidryas* larvae raised with or without predators. Therefore, only changes in vulnerability to larval predators should have contributed to differences in predation risk for different hatching phenotypes. Moreover, because early hatched tadpoles caught up in size quickly, they were likely similarly vulnerable after a short time, unless there were longer lasting induced changes in behavior or physiology. This may explain why initial differences between early and late-hatched tadpole survival disappeared as the experiment progressed under several different predation regimes.

Interestingly, though, compensatory growth itself may come with its own deferred costs such as reduced future growth, slowed sexual development, reduced physiological repair and maintenance, or depleted energetic reserves, though the specific costs will depend on the taxa and the mechanism of compensatory growth (Morgan and Metcalfe 2001; Mangle and Munch 2005). Food stress during a larval period can induce higher metabolic rates, lower investment in energy storage, and oxidative stress (Stoks et al. 2006; De Block and Stoks 2008). The costs incurred may come due immediately, or after a surprisingly long delay (Metcalfe and Monaghan 2001).

Summary

It is increasingly clear that understanding plasticity in the timing of life history switch points, including egg hatching, will be necessary to predict how organisms with complex life cycles fit into their larger ecological communities. Our study highlights that the consequences of hatching plasticity differ with predator community composition and/or diversity in subsequent life stages. A thorough understanding of the role of plasticity in ecological communities will require measuring the costs of plasticity over short- and long-term time frames, across multiple habitats, and in realistically complex experimental communities (e.g., Dahl et al. 2012).

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