

Adaptive plasticity in hatching age: A response to predation risk trade-offs

(life history/niche shift/*Agalychnis callidryas*)

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ABSTRACT The life histories of many animals are characterized by niche shifts, the timing of which can strongly affect fitness. In the tree frog *Agalychnis callidryas*, which has arboreal eggs, there is a trade-off between predation risks before and after hatching. When eggs are attacked by snakes, tadpoles escape by hatching rapidly and falling into the water below. Eggs not attacked by snakes hatch later, when newly emerged tadpoles are less vulnerable to aquatic predators. Plasticity in hatching allows embryos to use immediate, local information on risk of mortality to make instantaneous behavioral decisions about hatching and the accompanying shift from arboreal to aquatic habitats.

Most animals have complex life cycles. The most striking examples are species that undergo a metamorphosis, with dramatic morphological changes during development. However, changes in lifestyle, habitat, diet, and predators as an individual grows are far more widespread. These changes may be considered ontogenetic niche shifts (1).

Growth and mortality rates—the most important fitness components for prereproductive organisms—often vary with size. They also vary between habitats, and the ratio of mortality rate to growth rate may be used as a measure of habitat quality (1). If this ratio is lower in one habitat for small animals and in another habitat for larger animals, a niche shift during ontogeny may be advantageous. If growth and mortality rates vary within habitats, the best time or size to switch habitats may depend on environmental conditions (1–4). Niche-shift timing could evolve to some fixed optimum or, alternatively, show a plastic response to short-term environmental variation. Such plasticity has been demonstrated for amphibian metamorphosis (5–7) and habitat shifts in sunfish (8).

At hatching, animals become mobile and gain access to external food supplies. Their habitat and predators may also change, and mortality and growth rates may differ before and after hatching. In this context, hatching may be treated as an ontogenetic niche shift. To date, theoretical analyses of embryonic duration have concentrated on evolutionary change, not short-term phenotypic variation (9, 10). Clearly, much intraspecific variation in hatching age results simply from effects of temperature and other environmental factors on developmental rate (11, 12). However, a variable period of reduced or arrested embryonic development, often under conditions unfavorable for larval survival, is known in many species—e.g., see refs. 13–15. Embryonic duration may also vary while development continues, producing individuals that leave the egg at different sizes and stages of development (16–19). This variation can affect fitness because predation rates on hatchlings are often size- or stage-dependent (19–21). Based on niche-shift models—e.g., see refs. 1–4—we can predict that high mortality of hatchlings and a relatively safe egg (9) would favor delayed hatching. In contrast, high embryo mortality and enhanced posthatching growth rates would favor earlier hatching. Here, I show that in an amphibian, hatching age can influence the survival of embryos as well as of

hatchlings, and that environmentally cued hatching can reduce mortality.

I assessed the survival value of plasticity in hatching in the red-eyed tree frog, *Agalychnis callidryas*. *A. callidryas* eggs, which are laid on vegetation overhanging temporary ponds, are preyed on mainly by the cat-eyed snake, *Leptodeira septentrionalis*. At hatching the tadpoles fall into the water and are exposed to aquatic predators. My study had three parts. I measured development, predation, and hatching of egg clutches in the field. I experimentally determined the effects of snake attacks on hatching, and I tested the effect of hatching age on vulnerability to two common aquatic predators.

MATERIALS AND METHODS

Quantification of Natural Patterns. To assess patterns of development, predation, and hatching, I monitored individually marked clutches at natural ponds near Sirena Field Station in Corcovado National Park, Costa Rica. Numbered pieces of plastic flagging tape were tied to plants to mark clutch locations. Plastic cups were suspended under clutches to catch hatching tadpoles by the method of Hayes (22). Cups measured 9 cm in diameter by 7 cm deep and had small holes at a depth of 1 cm to prevent overflow of rainwater. Twice daily eggs were counted, developmental stage and clutch condition were noted, and any hatched tadpoles were counted. Hatchlings were released from the cups periodically (usually daily). There was no evidence of predation on tadpoles within the cups: hatched tadpoles left in cups during the day or overnight did not disappear. Incidence of egg predation was assessed on the basis of missing animals, corroborated with disturbance of egg jelly, and direct observations of predation. As a control for any effects of cups and flagging on predation rate, a series of unmarked clutches was also monitored through the prehatching period. Predation rates were identical on marked clutches and unmarked control clutches monitored simultaneously (19/39 marked; 15/30 unmarked; χ^2 test; $P = 0.9$). To assess the density of aquatic predators, I used a 0.5×0.5 m open-bottomed box to sample quadrats randomly located near the edges of one study pond where *A. callidryas* eggs are laid on vegetation overhanging the water.

Snake Effects on Hatching. To test if snake attacks induce hatching and if hatching is an effective escape mechanism for attacked embryos, I staged snake attacks on hatching age clutches. Young clutches were collected from ponds and were matched for age/developmental stage, pond of origin, and approximate clutch size. Experimental and control treatments were randomly assigned within pairs. Clutches were hung over containers of water in partitioned cages in the forest. Experimental cages contained a cat-eyed snake (*L. septentrionalis*) on the other side of the partition; control cages did not. Matched control and experimental clutches experienced the same conditions of temperature and rainfall. After eggs became capable of hatching, at 5 days of age and Gosner (23) stage 23, the partitions were removed. Snakes were observed and tadpoles and unhatched eggs were counted periodically

until the eggs either hatched or were eaten; observations were as frequent as every 15 min while snakes were active and eggs remained in experimental clutches, but there were never fewer than three observations in a day. For analysis, linear interpolation between the two adjacent data points was used to estimate the proportion of eggs hatched at times that fell between observations.

Vulnerability to Aquatic Predators. To determine if differences in hatching age affect vulnerability, I conducted predation experiments with newly hatched tadpoles of different ages and two predators: a shrimp, *Macrobrachium americanum*, and a fish, *Brachyraphis rhabdophora*. *M. americanum* is abundant in most *A. callidryas* breeding sites in Corcovado; *B. rhabdophora* is common in some breeding sites. Eggs and predators were collected from ponds, and predators were starved for 27–29 hours prior to use. Hatching was induced by mechanical disturbance of eggs, and hatchlings from 2 to 6 equal-aged clutches were pooled. Sets of tadpoles were drawn haphazardly from this pool and used in experiments within an hour of hatching. Experiments ran for 24 h and were replicated five times, except for one with small shrimp and 8-day-old hatchlings, which was replicated three times. Shrimp replicates used 12 tadpoles and 1 shrimp in a bowl with 3.25 liters of water filled to 2/3 of the water depth with leaf litter. Fish replicates used 10 tadpoles and one fish in 3.25 liters of water without leaf litter. Large shrimp were 28.8 ± 0.5 mm total length (mean \pm 1 SE), small shrimp were 20.8 ± 0.2 mm, and fish were 25.3 ± 0.5 mm.

RESULTS

Developmental rates were similar within and among the more than 1000 *A. callidryas* clutches observed at natural ponds and in the laboratory from 1991 to 1993. Embryos gastrulated the second morning after oviposition, and the neural folds developed later that day. The next morning they were in the tailbud stage. Muscular response had developed by the fourth morning after oviposition, and the heart began beating that day. At 4 days of age, embryos had well-developed gill circulation and transparent corneas. At 5 days of age, opercular development had begun, and embryos were capable of hatching. The only exceptions were a few embryos ($<1\%$, $n = 191$ clutches) that developed more slowly than their clutchmates, often showed morphological abnormalities, and sometimes died before hatching.

Despite the similarity of development rate, duration of the embryonic period varied greatly (Fig. 1). The first eggs hatched at 5 days of age, and the last, not until 10 days of age. Development and growth continued through the hatching period, such that tadpoles hatching later were more developed and larger than those hatching earlier. From age 5 to 7 days, tail length increased by 10–30% and embryos developed their external mouthparts (K.M.W., unpublished data).

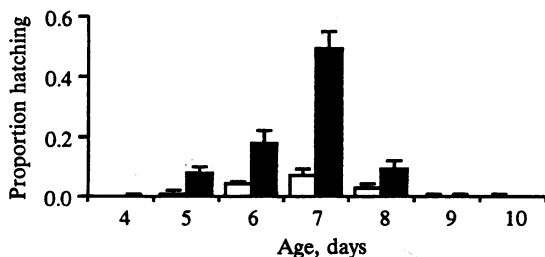


FIG. 1. Hatching pattern for undisturbed *A. callidryas* clutches in the field. Data are mean proportion hatched at each age ($+ 1$ SE) out of total hatch in each clutch ($n = 44$ clutches). Light bars indicate hatching during the day; dark bars indicate hatching at night. Depending on laying time, embryos turn 5 days old sometime during the "age 4" night.

Even after long embryonic periods tadpoles hatched with their gastrointestinal tracts filled with yolk. They did not begin feeding immediately upon hatching, and the delay before feeding was longer for animals that hatched earlier. Tadpoles that hatched at 8 days of age began feeding, as indicated by feces production, only 1 or 2 days after tadpoles that hatched 3 days earlier, at 5 days of age (K.M.W., unpublished data).

Snake attacks on *A. callidryas* eggs were common both before and during the hatching period. Before the onset of hatching, *L. septentrionalis* attacked 60% of monitored clutches and ate a total of 48% of the monitored eggs at one pond in 1991 ($n = 115$ clutches). At the same pond in 1992, they attacked 50.5% of clutches and ate 50% of the eggs ($n = 103$ clutches). Over the same developmental period at a second pond in 1992, *L. septentrionalis* attacked 27% of the clutches and ate 15% of the eggs ($n = 52$ clutches). As is evident from the differences in attack and egg-consumption data, snakes did not always consume all the eggs in the prehatching clutches they attacked. On first attacks, they consumed an average of 79% of a clutch ($\pm 2.9\%$ SE; $n = 106$ attacked clutches). The overall egg consumption rate includes repeated attacks on the same clutches. Of 113 clutches monitored through the hatching period in 1993, at the first pond, 41% were attacked by snakes. Polybid wasps were also occasional predators, attacking two monitored clutches at one pond in 1992.

Embryos in experimental clutches hatched rapidly when attacked by snakes (Figs. 2A and B and 3), whereas control clutches hatched gradually over a period of days (Fig. 2C). I conservatively compared maximal possible hatching duration—i.e., including the entire time intervals during which the first and last embryos hatched—for experimental clutches with minimal possible hatching duration—i.e., not including the intervals in which the first and last embryos hatched—for control clutches. Experimentals hatched more synchronously than did controls (Mann–Whitney U ; $P = 0.0002$). Median hatching time of experimental clutches was also earlier than

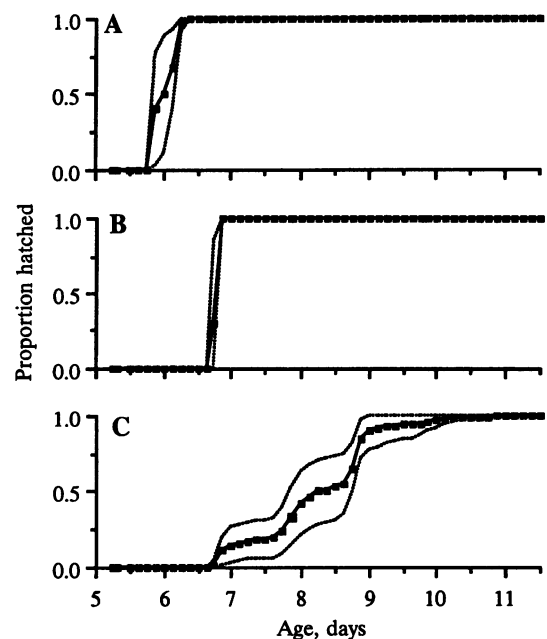


FIG. 2. Hatching of *A. callidryas* clutches with and without snake attack. Experimental clutches were attacked by *L. septentrionalis* at age 5 days (A; $n = 9$) or 6 days (B; $n = 5$); control clutches were not attacked (C; $n = 14$). Data are mean proportion hatched out of total hatch for each clutch. The 95% confidence intervals are shown by dotted lines. Time is plotted from midnight on the night of oviposition. Experimental clutches hatched rapidly when attacked by snakes; the mean hatching pattern reflects in part the variation in attack times. Embryos that hatched survived, and those that did not were eaten.



FIG. 3. *A. callidryas* embryos hatching upon attack by *L. septentrionalis*. Note the two newly hatched tadpoles on the egg jelly just below the snake's mouth and the lowest positioned embryo in the clutch which has ruptured the vitelline membrane and is about to exit the egg. Two more tadpoles, hatched moments before, have fallen part way down the leaf. The eggs are 6 days old.

that of controls (Mann-Whitney U ; $P = 0.0001$). Similarly, experimental clutches attached at 5 days of age hatched earlier than those attacked at 6 days of age (Mann-Whitney U ; $P = 0.0027$). By hatching, most embryos escaped from the snakes (74% mean escape rate $\pm 2.6\%$ SE; $n = 34$ clutches). Since on first attacks in the field snakes left only 21% of the eggs in clutches too young to hatch, hatching as a response to attack increases survivorship by a factor of 3.5. *A. callidryas* embryos hatch by vigorous movements that rupture the egg membrane. Individuals can hatch in seconds, and entire clutches in minutes; some embryos even hatched and escaped from eggs held in the mouths of snakes. Embryos that did not hatch when attacked were eaten.

Younger hatchlings were significantly more vulnerable to both shrimp and fish than were those hatched later (Fig. 4). The increase in survivorship with age is significant with both shrimp and fish predators, as are effects of the covariate, predator size (analysis of variance, shrimp predation, arcsine transformed data: age, $F_{3,32} = 4.629$, $P = 0.008$; shrimp size, $F_{1,32} = 62.937$, $P < 0.001$; fish predation: age, $F_{3,15} = 9.803$, $P = 0.001$; fish size, $F_{1,15} = 24.317$, $P < 0.001$). *M. americanum* are visibly abundant in most ponds where *A. callidryas* breeds in Corcovado. In one pond, the density of shrimp over 20 mm long was 8.4 ± 1.4 shrimp per m^2 ($n = 10$ quadrats) in 1992 and 15.2 ± 3.1 shrimp per m^2 in 1993 ($n = 20$ quadrats). *B. rhabdophora* co-occur with *A. callidryas* at several sites in Corcovado.

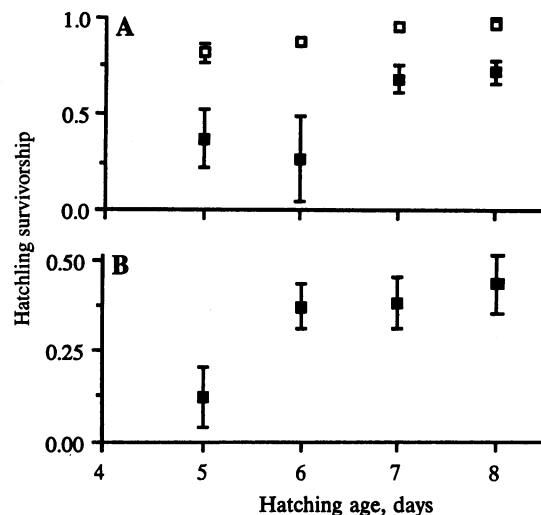


FIG. 4. Survivorship of *A. callidryas* hatchlings with shrimp, *M. americanum* (A), and fish, *B. rhabdophora* (B), in relation to hatching age. Data are mean proportion of tadpoles surviving (± 1 SE). In A, data from small shrimp (open symbols) and large shrimp (closed symbols) are plotted separately. The increase in survivorship with increasing hatching age is significant with both shrimp and fish predators, as is the effect of predator size.

DISCUSSION

Plasticity in the embryonic period of *A. callidryas* appears to increase fitness in the context of the predation risk trade-off at hatching. If attacked by an egg-eating snake, embryos hatch immediately and fall from the arboreal egg clutch into the water below, escaping from the snake. Clearly the ability to respond to snake attack by rapid hatching reduces mortality from snake predation. Early hatching entails a cost, however, because vulnerability of tadpoles to aquatic predators declines with age. The existence of an age effect on vulnerability to two very different predators—shrimp and fish—suggests that this effect may be more general; larger, more developed hatchlings are more likely to survive with a variety of predators (see also refs. 19–21). Thus, even if aquatic predator suites vary, in the absence of snake attack delayed hatching may be a good general strategy. In Corcovado, vulnerability of hatchlings to their most common predator, shrimp, declines from age 5 to 7 days (Fig. 4A), and most undisturbed embryos delay hatching until 7 days of age or later (Figs. 1 and 2C).

Tadpoles do not eat immediately upon hatching, and the delay before feeding begins is longer for younger hatchlings. This delay reduces the potential feeding benefits of early hatching and provides a simplified context in which to examine variation in risks. In Fig. 5, I present a simple graphical model of risk variation with age in two environments. Arboreal embryos cannot assess the presence or density of predators in the water, thus they cannot respond plastically to aquatic predators. However, lower mortality of tadpoles that hatch late could select for later hatching. Arboreal embryos can receive information about conditions, including snake attack, in their arboreal environment; this allows a condition-sensitive response. For that age range when the risk of mortality in the water falls between mortality risk in the egg under snake attack and mortality risk in the egg without snakes, a condition-sensitive hatching age is advantageous. The ability to hatch rapidly over a broad time period allows *A. callidryas* embryos to respond phenotypically to the actual and immediate risk of being eaten by a snake. For an individual, this is preferable to a fixed hatching time tuned to the historical likelihood of predation.

It might be argued that the hatching of *A. callidryas* in response to snake attack is simply an unmodified case of a

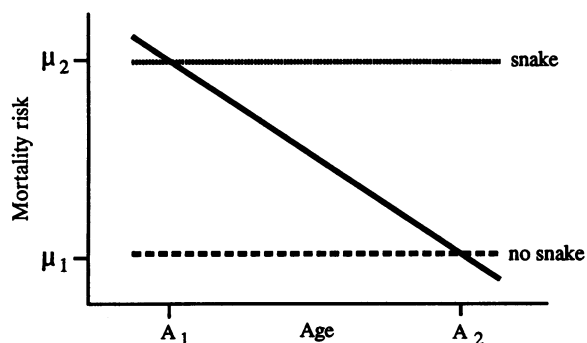


FIG. 5. Graphical model of mortality risks for *A. callidryas* through the hatching period. Instantaneous risk of mortality in the water (solid line) is a decreasing function of age, within some age range. Predation is the main source of tadpole mortality, and older animals are less vulnerable to aquatic predators. The level of mortality risk in arboreal eggs depends on the presence or absence of attacking snakes. In the absence of a snake the risk of mortality is low (μ_1 , dashed line); under snake attack the risk of mortality is consistently high (μ_2 , dotted line). Survival selection will favor different hatching ages depending on conditions in the egg. Eggs not attacked should hatch when mortality in the egg exceeds that in the water (A_2). Upon attack, risk of mortality in the egg rises from μ_1 to μ_2 , and if mortality risk in the water is lower, as it is after A_1 , embryos should hatch immediately. Thus, between A_1 and A_2 hatching plasticity and a rapid response to snake attack are advantageous.

more general response to mechanical disturbance. This appears not to be the case. The embryos of a congener, *Agalychnis spurrelli*, which shares breeding ponds and predators with *A. callidryas*, respond much less strongly to snake attack than do *A. callidryas* embryos; on average only 21% of the clutch escapes by hatching at an age when natural, undisturbed hatching has begun (K.M.W., unpublished data). The rapid hatching of young *A. callidryas* does not appear to be a general response to any type of disturbance. Two types of artificial stimulation, touching the eggs and moving the eggs by pulling at the jelly within which they are embedded, are ineffective at inducing early hatching. A third stimulus, touching and moving the eggs simultaneously by sliding forceps between the eggs into the jelly, is very effective at inducing rapid early hatching (K.M.W., unpublished data). Young eggs are also insensitive to a variety of other stimuli, including rain, wind, the vibration induced by foot transport, and earthquakes of up to 4.7 on the Richter scale (personal observation). Rapid hatching in *A. callidryas* is not a general response to all mechanical disturbance, nor is it exhibited by a closely related, ecologically similar species. It clearly increases fitness in the context of snake predation, but it is not possible with these data to argue that snake predation was the original selective force in the evolution of hatching plasticity and the rapid hatching response.

Early hatching as a means of escape from egg predators seems unusual and may be previously unknown in vertebrates. *A. callidryas*, however, are not the only embryos that respond to predators. A predator-induced delay in hatching has recently been demonstrated in another amphibian, the salamander *Ambystoma barbouri* (19), where it increases survival and appears not to have mortality or feeding costs (19, 24). Plasticity should be particularly valuable when the advantageous strategy under some conditions entails a cost under others, as for *A. callidryas*. Such trade-offs have been demonstrated for many predator-induced shifts in behavior and morphology (25–28). Phenotypic plasticity is increasingly being recognized as an important contributor to fitness in a

variable environment, as a target of selection, and as a factor in the evolution of organic diversity (28–30). The timing of hatching can be critical to survival, and plasticity at this niche shift can clearly be advantageous. The existence of such adaptive plasticity and the essentially behavioral nature of hatching make it an excellent system with which to study the ecology and evolution of life-history switch points (19). The conditions that make hatching plasticity advantageous in *A. callidryas*—different risks on either side of the niche shift and independent variation in those risks—are common, and such plasticity may also be widespread.

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1. Werner, E. E. & Gilliam, J. F. (1984) *Annu. Rev. Ecol. Syst.* **15**, 393–425.
2. Werner, E. E. (1988) in *Size-Structured Populations*, eds. Ebenman, B. & Persson, L. (Springer, Berlin), pp. 60–81.
3. Werner, E. E. (1986) *Am. Nat.* **128**, 319–341.
4. Rowe, L. & Ludwig, D. (1991) *Ecology* **72**, 413–427.
5. Newman, R. A. (1988) *Evolution* **42**, 774–783.
6. Crump, M. L. (1989) *Copeia* **1989**, 794–797.
7. Skelly, D. K. & Werner, E. E. (1990) *Ecology* **71**, 2313–2322.
8. Werner, E. E. & Hall, D. J. (1988) *Ecology* **69**, 1352–1366.
9. Shine, R. (1978) *J. Theor. Biol.* **75**, 417–424.
10. Nussbaum, R. A. & Shultz, D. L. (1989) *Am. Nat.* **133**, 591–603.
11. Blaxter, J. H. S. (1969) in *Fish Physiology: Reproduction and Growth, Bioluminescence, Pigments and Poisons*, eds. Hoar, W. S. & Randall, D. J. (Academic, New York), Vol. 3, pp. 177–252.
12. Duellman, W. E. & Trueb, L. (1986) *Biology of the Amphibians* (McGraw-Hill, New York).
13. Petranka, J. W., Just, J. J. & Crawford, E. C. (1982) *Science* **217**, 257–259.
14. Wourms, J. P. (1972) *J. Exp. Zool.* **182**, 389–414.
15. Saunders, D. S. (1982) *Insect Clocks* (Pergamon, Oxford).
16. Gollmann, B. & Gollmann, G. (1992) *Zool. Anz.* **229**, 191–199.
17. Williamson, I. & Bull, C. M. (1989) *Copeia* **1989**, 349–356.
18. Cumming, R. L. (1993) *Bull. Mar. Sci.* **52**, 760–771.
19. Sih, A. & Moore, R. D. (1993) *Am. Nat.* **142**, 947–960.
20. Kaplan, R. H. (1992) *Ecology* **73**, 280–288.
21. Petranka, J. W., Sih, A., Kats, L. B. & Holomuzki, J. R. (1987) *Oecologia* **71**, 624–630.
22. Hayes, M. P. (1983) *Herp. Rev.* **14**, 115–116.
23. Gosner, K. L. (1960) *Herpetologica* **16**, 183–190.
24. Sih, A. & Maurer, E. (1992) *J. Herpetol.* **26**, 116–120.
25. Sih, A. (1987) in *Predation: Direct and Indirect Impacts on Aquatic Communities*, eds. Kerfoot, W. C. & Sih, A. (Univ. Press New England, Hanover, NH), pp. 203–224.
26. Lima, S. L. & Dill, L. M. (1990) *Can. J. Zool.* **68**, 619–640.
27. Harvell, C. D. (1986) *Am. Nat.* **128**, 810–823.
28. Travis, J. (1994) in *Ecomorphology: Integrative Organismal Biology*, eds. Wainwright, P. C. & Reilly, S. (Univ. Chicago Press, Chicago), pp. 99–122.
29. Travis, J. (1994) in *Ecological Genetics*, ed. Real, L. A. (Princeton Univ. Press, Princeton), pp. 171–204.
30. West-Eberhard, M. J. (1989) *Annu. Rev. Ecol. Syst.* **20**, 249–278.