



# Wasp predation and wasp-induced hatching of red-eyed treefrog eggs

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Eggs often suffer high levels of predation and, compared with older animals, embryos have few options available for antipredator defence. None the less, hatchlings can escape from many predators to which eggs are vulnerable. I studied early hatching as an antipredator defence of red-eyed treefrog embryos, *Agalychnis callidryas*, in response to egg predation by social wasps (*Polybia rejecta*). Red-eyed treefrogs attach their eggs to vegetation overhanging water, where they are exposed to arboreal and aerial predators. Wasps attacked half the egg clutches and killed almost a quarter of the eggs I monitored at a natural breeding site in Panama. Hatching tadpoles fall into the water, where they face aquatic predators. As predicted from improved survival of older hatchlings with aquatic predators, most undisturbed eggs hatched relatively late. However, many younger embryos directly attacked by wasps hatched immediately. Embryos attacked by wasps hatched as much as a third younger than the peak undisturbed hatching age, and most hatching embryos escaped. Thus hatching is an effective defence against wasp predation, and plasticity in hatching stage allows embryos to balance risks from stage-specific egg and larval predators. Wasp-induced hatching is behaviourally similar to the snake-induced hatching previously described in *A. callidryas*, but occurs in fewer eggs at a time, congruent with the scale of the risk. Individual embryos hatch in response to wasps, which take single eggs, whereas whole clutches hatch in response to snakes, which consume entire clutches. Embryos of *A. callidryas* thus respond appropriately to graded variation in mortality risks.

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The transition from embryonic to larval stages can be considered in the same theoretical context as other life history switch points, such as metamorphosis. The timing of these ontogenetic niche shifts should vary with the costs and benefits accruing in each stage (Werner & Gilliam 1984; Werner 1986, 1988). The costs and benefits depend on both environmental conditions and the organism's phenotype (e.g. size). Specifically, animals should respond to increased risk or decreased benefits in the first stage by accelerating the transition, and to increased risk or decreased benefits in the second stage by delaying the transition.

Amphibian embryos of several species alter hatching with changes in predation risk as predicted. For instance, streamside salamanders, *Ambystoma barbouri*, delay hatching in response to cues from larval predators (Sih & Moore 1993; Moore et al. 1996). Red-eyed treefrogs, *Agalychnis callidryas*, hatch early to escape from attacks by egg-eating snakes (Warkentin 1995). The reed frog *Hyperolius cinnamomeoventris* emerges at a smaller size

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from clutches infested with predatory fly larvae (Vonesh 2000), and *Rana cascade* and *Hyla regilla* hatch early in response to egg-eating leeches (D. P. Chivers, personal communication). Here I use direct observations of predation in nature to examine the response of red-eyed treefrog embryos to an invertebrate egg predator, and find escape hatching behaviour similar to that shown with egg-eating snakes.

The red-eyed treefrog inhabits lowland wet forest from the Yucatan through Panama. It attaches its eggs to vegetation overhanging water, usually seasonal ponds or swamps, and upon hatching the tadpoles fall into the water. Thus hatching involves a habitat shift, and there is a trade-off of risks between egg and larval stages. Arboreal and aerial predators attack eggs, but have no access to tadpoles, while aquatic predators prey on tadpoles but have no access to eggs. Although development rate varies remarkably little within populations, hatching age varies substantially (Warkentin 1995, 1999a). Undisturbed embryos hatch late, when they are more developed and better able to escape from predatory fish and shrimp (Warkentin 1995, 1999b). If attacked by egg-eating snakes, however, relatively young embryos

hatch immediately, and hatching is an effective means of escape from snakes (Warkentin 1995).

The majority of predation on *A. callidryas* eggs at two sites in Costa Rica is by snakes, mostly *Leptodeira septentrionalis* (Corcovado National Park: Warkentin 1995; La Selva Biological Station: unpublished data). None the less, snakes are not the only agents of egg mortality. Wasps and monkeys prey on the eggs, an egg-killing fungus can infect the clutches, and fly larvae may also kill eggs (Villa 1979; Villa & Townsend 1983; Warkentin 1995, unpublished data). Life history theory predicts that, all else being equal, any increased risk in the life stage before a transition point should accelerate the transition (Werner & Gilliam 1984; Werner 1986, 1988). To the extent that hatching provides an effective defence against an egg-specific risk, selection should favour hatching in response to that risk. However, the high mortality of young hatchlings due to aquatic predators selects against unnecessary early hatching. Depending on the cues available from egg predators and other sources of egg mortality, selection against early hatching could limit the expression of the escape hatching response.

*Polybia* (Vespidae, Polistinae, Epiponini) are successful and abundant swarm-founding social wasps that are reported to prey on soft-bodied arthropods, especially lepidopteran larvae (Jeanne 1991; Raveret Richter & Jeanne 1991). *Polybia* also prey on *A. callidryas* eggs in all three locations where I have studied this frog (La Selva Biological Station and Corcovado National Park, Costa Rica, and Gamboa, Panama). *Polybia rejecta* occurs through Central America and into South America (Richards 1978), overlapping broadly in distribution with *A. callidryas*. Here I measure the intensity of predation on *A. callidryas* eggs by *P. rejecta*, then test whether wasp attack induces early hatching and assess the effectiveness of hatching as a defence against wasps.

## METHODS

### Study Site

All clutch monitoring and observation was conducted at Ocelot Pond, 2 km south of Gamboa in Soberanía National Park, Republic of Panama, during June–August 1998, under permits from the Panamanian Institute of Natural Renewable Resources.

### Monitoring Egg Clutches

To assess rates and patterns of predation and hatching I monitored 123 individually marked egg clutches of *A. callidryas* at Ocelot Pond in two series, June 10–16 and July 19–27, 1998 (52 and 71 clutches, respectively). I located clutches the morning after oviposition and marked each clutch site with a numbered plastic flag tied to the vegetation, as in Warkentin (1995). Before embryos became capable of hatching, I hung a plastic cup under each clutch to catch hatchlings (Hayes 1983; Warkentin 1995). I checked clutches daily or, once hatching began, twice daily. At each observation I counted eggs and

hatchlings, and noted the condition of the clutch and the developmental stage of eggs. I released hatchlings from cups periodically, usually daily. As in Warkentin (1995), there was no evidence of predation on hatchlings in cups.

I assessed egg predation rates based on missing animals, corroborated with evidence of disturbance of the clutch and direct observations of predation. Based on direct observations of attacks by snakes (Warkentin 1995) and wasps, the patterns of egg loss and other damage caused by these predators are distinctive. Snakes take entire clutches or large portions of embryos and jelly on a single visit, leaving any remaining eggs undamaged. Wasps take one embryo at a time in repeated visits, and frequently damage additional embryos, but leave the jelly (see Results for details). When I did not have direct observations of predators I used the pattern of damage and egg loss to determine whether a snake, wasp, or other unknown predator had taken eggs. In 23 months of field work on *A. callidryas* eggs in Costa Rica and Panama since 1991 I have seen no evidence that any nonwasp predator produces clutch damage similar in appearance to the damage caused by wasps.

As one test of the effect of wasp attack on hatching, I calculated the proportion of hatchable embryos present at the start of each age interval (between observations) that hatched in that interval, for each clutch. I considered embryos that were dead, developmentally abnormal or injured to be not hatchable, as well as those younger than 4 days. I then compared the proportion that hatched from clutches that were undisturbed versus clutches that were attacked by wasps during each age interval. I determined wasp attacks during an interval based on direct observations of wasps on clutches or floating in cups, and new clutch damage characteristic of wasps. I considered clutches to be undisturbed if I had not directly observed disturbance, there were no missing or newly damaged eggs, nor was there any other sign of new disturbance (e.g. leaf displaced from the cup).

To assess whether wasps mainly attack clutches containing dead eggs (i.e. may simply be attracted to carrion rather than to live frog eggs), I determined the frequency of initial attacks on clutches containing dead eggs and on those containing only live eggs. I assumed that the condition of a clutch when the first wasp attack occurred was the same as in the last observation prior to attack. I excluded one clutch that began hatching before the initial attack, because it is unclear whether the capsules left by hatching embryos are perceived as carrion.

### Direct Observations of Attacks

To determine the fine temporal pattern of hatching in relation to wasp attacks, and assess the effectiveness of hatching as a defence, I directly observed natural wasp attacks on *A. callidryas* clutches. I continuously observed 4- and 5-day embryos for up to several hours per clutch during the day on 31 May and 9–12 June 1998 (2335 total clutch-min observed, mean  $\pm$  SE =  $97 \pm 16$  min per clutch,  $N=24$  clutches). *Agalychnis callidryas* embryos from this population cannot hatch before 4 days of age (develop-

mental stage 23, Gosner 1960) and at 5 days, all embryos are capable of hatching but are still underdeveloped. Most undisturbed eggs hatch during the night they turn 7 days old. Some clutches were observed at both 4 and 5 days, and some nearby clutches were observed concurrently. I recorded all incidences of attack, mortality and hatching, and periodically counted eggs to corroborate my observations. During these observation periods I saw 35 wasp attacks on focal clutches.

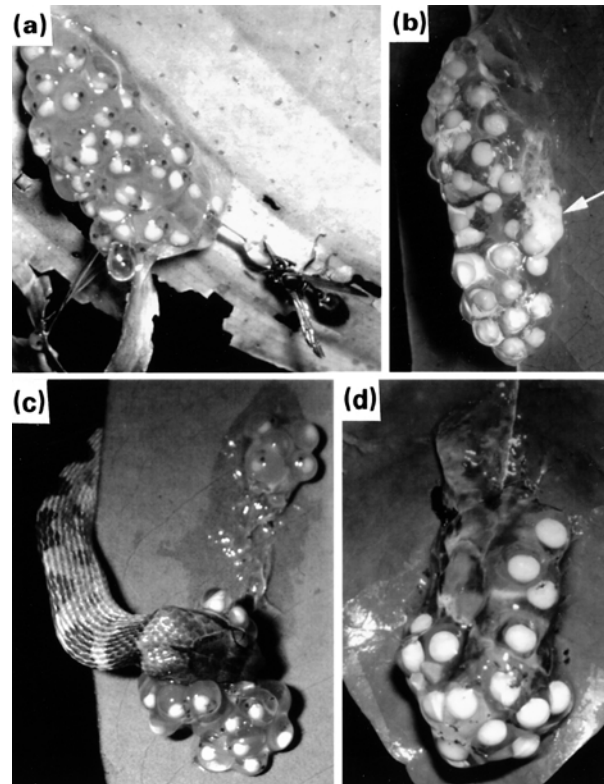
I defined an attack on a clutch to occur when a wasp landed on or beside the clutch and grasped and pulled at one or more eggs, regardless of the final outcome. Wasps that landed on and walked over clutches without pulling at eggs were not considered to be attacking. I made no attempt to quantify the number or response of eggs that were merely touched by wasps, although some may have been grasped briefly, in passing. Individual eggs were considered attacked when a wasp clearly pulled at the egg. I defined hatching to occur when an embryo exited the egg capsule under its own power. Whenever possible, I distinguished between hatching of individual eggs attacked by wasps and hatching during an attack of eggs that were not directly attacked, but adjacent to attacked eggs.

To assess the immediate hatching response of embryos to wasp attack, I calculated the hatching rate (tadpoles/min) of clutches while undisturbed and while attacked. For the 'attacked' hatching rate, I included the period while the wasp was on the clutch manipulating eggs and any hatching that occurred immediately after the wasp left, up to a maximum total period of 3 min per attack (mean 1.8 min, 35 attacks). No attacks I observed on hatching-age clutches were longer than 3 min. To calculate the 'undisturbed' hatching rate I excluded only 3-min periods during and immediately after wasp attacks. Because not all clutches contributed both attacked and undisturbed data, I tested the difference in hatching rates with an unpaired test.

To assess the effectiveness of hatching as a means of escape, I compared the proportion of embryos that survived in each clutch of those that hatched when their egg was attacked by a wasp, versus those that did not hatch under attack. I only used cases where the position of the clutch and the wasp allowed me to clearly see hatching from eggs directly grasped by the wasp ( $N=18$  clutches, 43 eggs). I excluded embryos that hatched, or might have hatched, out of eggs adjacent to those under direct attack. I also excluded mortality of embryos less developed than their siblings, as these may have been incapable of hatching. As above, I used an unpaired test.

During the course of clutch monitoring (above) and other field work, I opportunistically watched over 70 additional wasp attacks on *A. callidryas* clutches of various ages during June–August 1998. I used both these opportunistic observations and the 35 attacks that occurred during the periods of observation of 4- and 5-day clutches to identify patterns of clutch damage characteristic of wasp predation.

Voucher specimens of wasps are deposited at the Smithsonian Tropical Research Institute, Panama (*P. rejecta* from Ocelot Pond) and at the Instituto Nacional



**Figure 1.** Wasp and snake predation on eggs of *A. callidryas*. (a) A wasp (*Polybia* sp.) pulling a near-hatching stage egg from a clutch, Corcovado National Park, Costa Rica. (b) A young clutch with some eggs damaged by wasps (*P. rejecta*), Ocelot Pond, Panama. Note the broken and smeared yolks (arrow), but basically intact jelly. (c) A snake (*L. septentrionalis*), consuming near-hatching stage eggs, Corcovado. The snake takes several eggs at a time, removing both embryos and jelly. (d) A young clutch partially eaten by a snake, Corcovado. Note the cleanly torn jelly and absence of damaged eggs.

de Biodiversidad, Costa Rica (wasps from Corcovado and La Selva).

## RESULTS

### Wasp Attacks and Patterns of Damage

Wasp attacks on red-eyed treefrog eggs followed characteristic patterns, and the damage they caused to egg clutches was clearly distinguishable from that caused by egg-eating snakes (Fig. 1). Wasps attacked and removed embryos from a clutch one at a time. The type of damage varied depending on how easily eggs detached from the clutch, and how developed the embryos were. Attacking wasps grasped individual eggs with their mouthparts and pulled (Fig. 1a). They extracted embryos from their egg capsules, often after removing the egg from the clutch. With well-developed embryos that struggled, the wasps dragged the embryos around on the leaf and bit them, presumably to subdue them. Finally the wasps carried the embryos away. Clutches attacked by wasps usually experienced repeat attacks, losing one embryo at a time over hours or days. If an egg did not

detach from a clutch easily, the wasp sometimes broke the vitelline membrane but left the embryo inside it. Some of these embryos hatched later but many died from injury or dehydration. With young embryos, wasps often broke the yolk and were able to extract only part of the embryo. They sometimes ingested some yolk immediately, or carried away pieces of tissue, but they left some of the broken yolk in the remains of the capsule and smeared on the jelly and leaf (Fig. 1b). With more developed embryos, wasps sometimes left severed body parts stuck to the clutch or leaf. Older, hatchable embryos that escaped sometimes had cuts on their body or tail, or a piece of the tail missing. Wasps that successfully extracted an embryo or part thereof that could be carried left with it soon after. If, as often happened with young eggs, a wasp failed to extract cohesive, transportable tissue, it often broke a series of eggs in one visit to the clutch. Similarly, wasps that failed to capture hatching embryos often attacked a series of eggs in one visit.

A high proportion of clutches attacked by wasps were completely healthy, containing no dead embryos prior to the initial attack (62%,  $N=60$ ). In no case did the condition of newly attacked, previously healthy clutches suggest that damage from an additional, nonwasp source had also occurred in the interval between observations. These clutches contained no desiccated or squashed eggs, no fungal hyphae, and no fly larvae; there were only healthy eggs and the missing and injured embryos characteristic of wasp predation. Of clutches not attacked by wasps, 73% ( $N=62$ ) contained dead eggs at some point during monitoring, suggesting that egg carrion is readily available. Furthermore, I observed *P. rejecta* on clutches containing dead embryos, often killed in prior attacks, to touch and reject this carrion then move on to attack live eggs.

### Fates of Embryos

Less than half of the eggs I monitored hatched successfully (Table 1). Of eggs that did not survive to hatching, the largest proportion were killed by wasps, and a substantial number fell prey to snakes. The remaining eggs perished from a variety of causes. Some were unfertilized or showed developmental abnormalities and then died. During a period without rain some died from desiccation. Later, during heavy rains, some drowned when pond levels rose or leaves fell into the water. Small numbers of embryos met a variety of other fates, shown in Table 1.

### Hatching Patterns

In undisturbed clutches, embryos hatched gradually over a period of days (Fig. 2). They became capable of hatching at 4 days old, but most delayed hatching until the night they turned 7 days old.

To assess the effect of wasp predation on hatching, I compared hatching rates of eggs in wasp attacked (A) versus undisturbed (U) clutches during each age interval. Eggs in clutches attacked by wasps hatched at a higher rate than those in undisturbed clutches (Fig. 3). Sample

**Table 1.** Fates of *A. callidryas* embryos at Ocelot Pond, Panama

| Fate                        | Clutches affected | Proportion of embryos |
|-----------------------------|-------------------|-----------------------|
| Hatched                     | 0.634 (78)        | 0.424±0.036           |
| Killed by wasps*            | 0.496 (61)        | 0.235±0.031           |
| Eaten by snakes             | 0.236 (29)        | 0.189±0.033           |
| Failed to develop normally† | 0.285 (35)        | 0.036±0.012           |
| Drowned                     | 0.049 (6)         | 0.036±0.016           |
| Desiccated‡                 | 0.089 (11)        | 0.032±0.013           |
| Unknown predator§           | 0.089 (11)        | 0.014±0.007           |
| Other dead**                | 0.041 (5)         | 0.024±0.012           |
| Other live††                | 0.033 (4)         | 0.012±0.007           |

Data are the proportion (number) of clutches affected, and the mean±SE proportion of embryos per clutch, from all clutches monitored. Sample size is 123 clutches except for the proportion of embryos hatched and preyed on by snakes and wasps, where it is 122. One clutch was attacked by wasps before the first egg count, and a snake displaced one hatchable clutch from above its cup.

\*Includes both embryos taken by wasps and those killed but left on the clutch.

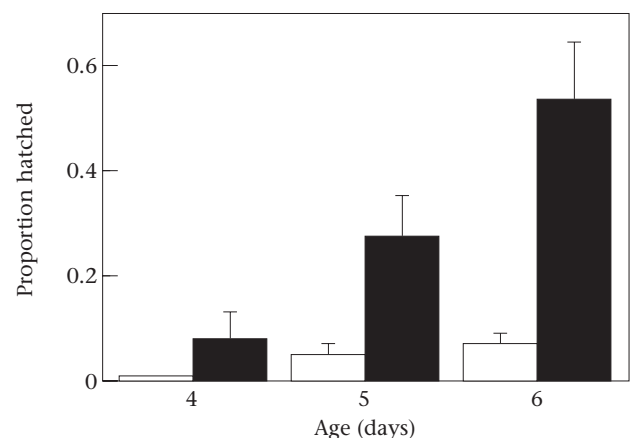
†Includes eggs that never developed and may not have been fertilized, and embryos that developed abnormally and then died.

‡Includes embryos in three clutches that died after their clutch was disrupted and partially eaten by a snake.

§Could include some predation by wasps on well-developed eggs.

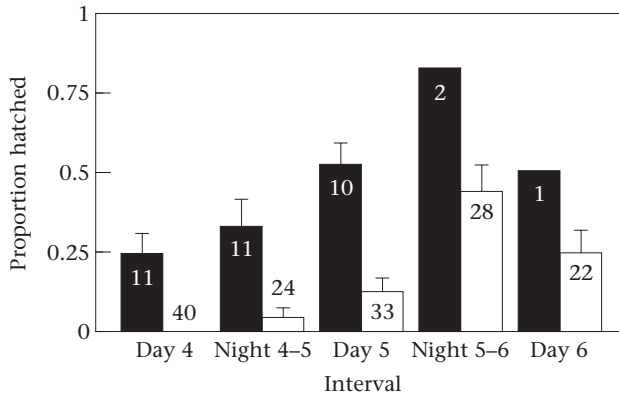
\*\*Includes embryos killed by fungus in two clutches, a clutch that fell on the ground when a caterpillar severed its leaf, a clutch squashed between two leaves by a snake feeding on another nearby clutch, and embryos that died in a clutch infested with fly larvae.

††Includes hatchable eggs that fell into the water, which typically hatch, and missing embryos (presumed hatched and escaped) from a clutch whose cup was completely flooded.

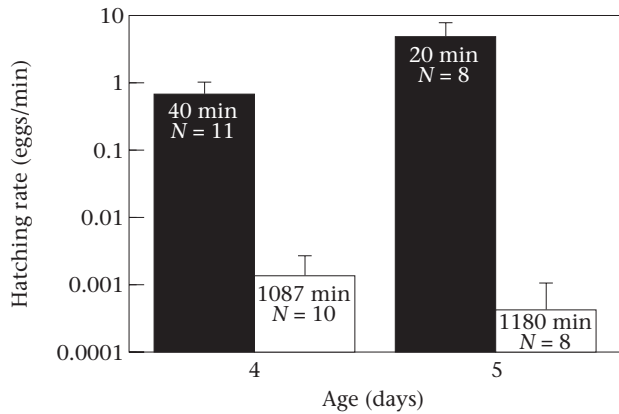


**Figure 2.** Hatching pattern of healthy, never disturbed *A. callidryas* clutches at Ocelot Pond, Panama. Data are the mean±SE proportion hatched in each interval out of the total hatched in each clutch ( $N=14$ ). □: Hatched during the day; ■: hatched at night. Depending on laying time, embryos turn 7 days old sometime during the 'age 6' night.

sizes of attacked clutches permitted statistical tests on day 4, night 4–5 and day 5. In all cases the higher hatching rate in attacked clutches was highly significant (Mann–Whitney  $U$  test: day 4:  $U=7.5$ ,  $N_U=40$ ,  $N_A=11$ ,  $P<0.0001$ ; night 4–5:  $U=17$ ,  $N_U=24$ ,  $N_A=11$ ,  $P<0.0001$ ; day 5:  $U=36$ ,  $N_U=33$ ,  $N_A=10$ ,  $P=0.0002$ ; Bonferroni corrected  $\alpha=0.017$ ).

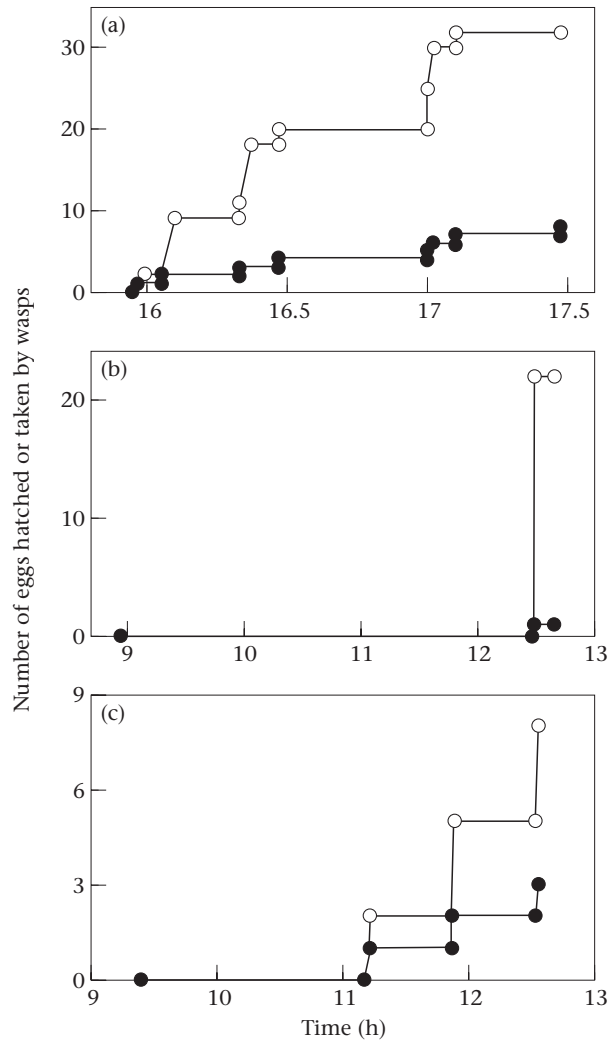


**Figure 3.** Mean+SE proportion of *A. callidryas* embryos hatching at different ages from clutches that were attacked by wasps (■) or undisturbed (□) during each interval. Data are the proportion of live, normally developed embryos present at the start of an interval that hatched during that interval. Number of clutches is indicated for each bar. A higher proportion of attacked than undisturbed embryos hatched in each interval, especially at younger ages.



**Figure 4.** Hatching rates of *A. callidryas* eggs while undisturbed (□) or under attack by wasps (■) at different ages. Data are means +SE. Total clutch-minutes observed and number of clutches are indicated. Embryos become capable of hatching sometime during day 4.

My direct observations of wasp predation and hatching indicate that hatching was closely associated with wasp attacks. The hatching rate under attack was roughly three orders of magnitude higher than the hatching rate of undisturbed clutches (Fig. 4). It was significantly higher at 5 days but not 4 days (Mann-Whitney *U* test: day 5:  $U=64$ ,  $N_A=N_U=8$ ,  $P=0.0005$ ; day 4:  $U=71.5$ ,  $N_A=11$ ,  $N_U=10$ ,  $P=0.12$ ; Bonferroni corrected  $\alpha=0.025$ ). In four of 11 clutches, 4-day eggs hatched when attacked (10 embryos), and one unattacked 4-day egg hatched. Five-day-old embryos rarely hatched if undisturbed, but hatched readily under attack. In 5-day clutches, both embryos in eggs directly grasped by the wasp and those in adjacent eggs hatched under attack, while eggs in other parts of the clutch did not hatch. Wasps that failed to capture hatching embryos frequently attacked adjacent eggs.



**Figure 5.** Pattern of wasp predation (—●—) and hatching (—○—) in three continuously observed *A. callidryas* clutches (a, b, c). All clutches were 5 days old; time is measured from midnight between day 4 and 5.

Figure 5 illustrates the pattern of hatching and predation for three 5-day clutches. All hatching was closely associated with wasp attacks. For instance, in clutch 4 (Fig. 5a), at 1700 hours a wasp grabbed an egg, which hatched. It then grabbed another, which hatched, and a third which also hatched. The wasp caught the fourth embryo, and two adjacent eggs hatched. The first wasp left and at 1701 hours a second wasp arrived. Again, the first three embryos grabbed by the wasp escaped by hatching. The wasp caught the fourth embryo and two additional, adjacent eggs hatched.

From the continuous clutch observations, the proportion of embryos that survived direct attacks was significantly higher for embryos that hatched (H), versus those that did not hatch (Mann-Whitney *U* test:  $U=8$ ,  $N_H=5$ ,  $N_{noTH}=15$ ,  $P=0.0007$ ). Of 17 embryos that hatched when attacked, 15 escaped (88%). The two that died did not fall into water, but rather onto a solid substrate from which the wasp retrieved them. Of 26 embryos that did

not hatch from eggs directly attacked by wasps, 24 were killed (92%). These embryos were extracted from the egg by the wasp, and their subsequent struggles failed to free them. Two other eggs were broken, but the wasp did not extract the embryos. Although I considered these embryos 'alive' for the statistical test of survival rates, such embryos often died from injuries or desiccation.

## DISCUSSION

### Wasp Predation on Frog Eggs

I observed high levels of predation on red-eyed treefrog eggs by the social wasp *Polybia rejecta*. *Polybia* are typically considered to be predators of soft-bodied arthropods, especially lepidopteran larvae (Jeanne 1991; Raveret Richter & Jeanne 1991). They have not been previously described to prey on vertebrates, and in general wasp predation on vertebrates is rare (Raveret Richter 2000). This study demonstrates that wasps can be significant predators of anuran embryos, and suggests that anuran embryos may be locally important food sources for certain wasps.

There are a few other records of wasp predation on frog eggs. Lacey (1979) reported large numbers of *Angiopolybia pallens* (Vespidae, Polistinae, Epiponini) preying on eggs and tadpoles in the terrestrial foam nests of the frog *Leptodactylus pentadactylus* in Brazil. I observed *Agelais* preying on *A. callidryas* eggs and on the arboreal eggs of *Hyla ebraccata* in Costa Rica. I also recorded *Polybia*, possibly *P. rejecta*, preying on both *A. callidryas* and *A. saltator* eggs in Costa Rica. Furthermore, I observed occasional predation on *A. callidryas* eggs at Ocelot Pond by a second wasp species (also Epiponini, three observations). Finally, McDiarmid (1978) noted predation by an unidentified wasp on the arboreal eggs of the glass frog *Centrolenella colymbiophyllum* in Costa Rica (now *Hyalinobatrachium colymbiophyllum*, Ruiz-Carranza & Lynch 1991). Thus it appears that several species of wasps prey on frog eggs, and embryos of several species of frogs fall prey to wasps.

In my observations of frog egg predation by *Agelais*, the clutches were already damaged by desiccation or by *Polybia*, and contained dead eggs. In Lacey's (1979) observations the clutches contained fly larvae, which also kill frog eggs. This is consistent with prior reports that *Agelais*, and other epiponines, are attracted to carrion (Jeanne et al. 1995; O'Donnell 1995). In contrast the *Polybia* in both Panama and Costa Rica frequently attacked healthy, undamaged clutches, and *P. rejecta* appeared to prefer live embryos as prey in clutches where dead eggs were also present. Thus predation on frog eggs by these wasps is not just a particular expression of a general attraction to carrion; they appear specifically attracted to live frog eggs.

For wasps living near the breeding sites of frogs with terrestrial or arboreal egg deposition, frog embryos offer an easily exploited source of protein and energy. *Agalychnis callidryas* embryos are a convenient size to transport, require minimal processing, and occur in clumps (mean 40 eggs/clutch, Warkentin 1995). They are

physically vulnerable, being incapable of fighting or, at least early in development, of fleeing. Like most embryos (Orians & Janzen 1974), and in contrast to many lepidopteran larvae (e.g. Boppré 1990; Dyer 1995), they appear to be chemically undefended. Oviposition sites are spatially predictable, because the eggs are deposited on vegetation overhanging water. Especially in deep ponds with fringing vegetation but little emergent vegetation, like Ocelot Pond, potential oviposition sites are spatially constrained. Thus new egg clutches are deposited at or near previously used oviposition sites throughout the rainy season, facilitating their detection by predators, like wasps, that remember prey locations (Raveret Richter & Jeanne 1985, 1991; Raveret Richter 2000). Arboreal frog eggs may be an important resource for wasp colonies near ponds.

### Wasp-induced Hatching

Hatching is an effective defence against wasp predation. Once capable of hatching, *A. callidryas* embryos hatch rapidly in response to attack by wasps. Early hatching is, therefore, not a response that is specific to snake attack but rather appears to be a broader strategy that embryos employ when faced with a high risk of mortality in the egg.

Embryos 5 days and older frequently hatch when attacked, and day 4 embryos sometimes hatch when attacked. Two factors may explain the lower hatching rate of the younger embryos. First, depending on the time of oviposition and attack, some attacked 4-day embryos may not be developmentally competent to hatch. Second, the youngest hatchable embryos are also the most vulnerable to aquatic predators, which reduces the benefit of early hatching (Warkentin 1995, 1999b).

Although I opportunistically observed wasp attacks on clutches of all ages, I detected few attacks on clutches older than 5 days in the monitored series of clutches. This may be a methodological artefact, rather than reflecting a difference in actual attack rates. In any particular clutch, if all embryos attacked by a wasp hatched and escaped undamaged, the attack would be undetectable by indirect methods. Older eggs hatch more readily than younger eggs, thus attacks between observations may be more likely to go undetected when clutches are older.

Wasp-induced hatching appears similar to snake-induced hatching, but on a smaller scale. In both cases embryos respond to direct physical disturbance of eggs with vigorous movements that rupture the egg envelope and propel them from it. In snake attacks, entire clutches can hatch in minutes (Warkentin 1995). In wasp attacks, only a few eggs hatch (those eggs grasped by the wasp, and sometimes their immediate neighbours). Since snakes can consume entire clutches, all eggs in an attacked clutch are at risk. Thus mass hatching appears adaptive. Wasps can only carry away one embryo at a time, and cease their attack once they have acquired a transportable prey item. Thus not all eggs in an attacked clutch are at risk and, appropriately given the cost of early hatching, eggs distant from the site of attack do not hatch. Eggs grasped by the wasp are most at risk, and

most likely to hatch. Neighbouring eggs face intermediate risk; if the embryo in the grasped egg escapes, the wasp then turns to the next closest egg. Not surprisingly, these nearby eggs sometimes hatch before they are directly attacked.

As with snakes, hatching is an effective defence against wasps. In the attacks that I observed, all hatching tadpoles that fell into water escaped. In contrast all embryos that failed to hatch and instead allowed wasps to extract them from the egg were killed. Because wasps occasionally attack eggs but fail to extract the embryo, and some of these embryos survive, the mortality of attacked eggs that do not hatch is not 100%. Furthermore, injuries indicate that some hatchlings escape from the grasp of a wasp. These animals may be grasped briefly as they exit the egg under their own power. Alternatively, although I never observed this, it is possible that some are extracted by wasps and then escape. None the less, for *A. callidryas* embryos, the chance of surviving a wasp attack by hatching is clearly better than gambling that the wasp will abandon the attack, or struggling only after being extracted by the wasp.

### Cues to Mortality Risk and Embryonic Decision Making

*Agalychnis callidryas* embryos hatch early in response to at least four different natural risks: wasp attack, snake attack (Warkentin 1995), fungus infestation and flooding (unpublished data). They also hatch in response to some artificial stimuli including manual egg jiggling and hypoxia (Warkentin 1995, unpublished data). The embryos are, however, unresponsive to disturbance by earthquakes, tropical storms, and the collecting and transportation of egg clutches (Warkentin 1995). This pattern of sensitivity suggests that, in deciding when to hatch, embryos use cues in multiple sensory modalities, and distinguish among different types of mechanical cues.

Immediate, relatively synchronous hatching of small groups of eggs or entire clutches occurs in response to physical disturbance by wasps and snakes, and manual egg jiggling. In contrast, fungus, flooding and hypoxia induce more gradual early hatching. Thus mechanical stimulation is sufficient and appears to be necessary for rapid, synchronous hatching. The displacement of eggs in wasp attacks is substantially less than that in snake attacks, and the force is directly applied to a much smaller portion of the clutch. Wasps also induce less hatching than snakes. This is consistent with a hatching response that is graded in a simple way with stimulus intensity. The hatching of embryos directly attacked by wasps while distant clutchmates fail to hatch could also be mediated by stimulus intensity. However, the lack of hatching in tropical storms with high winds and rainfall of up to 26 cm/day (enough to knock eggs from clutches; personal observation) is not consistent with a simple, intensity-mediated response to physical disturbance. Thus in assessing risk embryos must distinguish between mechanical cues from different sources. They might use frequency information or the temporal pattern of stimu-

lation, as well as amplitude. Vibrational cues surely differ substantially between snake attacks on clutches attached to stiff branches, versus clutches attached to leaves with flexible petioles, but this variation is not relevant to risk assessment. The properties of the egg jelly must affect the transmission of vibration to embryos, which may provide a means to distinguish direct contact with the clutch (i.e. a predator pulling at eggs) from less risky types of non-contact disturbance, such as earthquakes and storms. Investigation of the vibrational cues provided by different predators and benign disturbances and the response of embryos to such cues would be worthwhile.

The hatching of *A. callidryas* embryos in response to fungus, flooding and hypoxia indicates that mechanical cues are not the only relevant stimuli. Oxygen stress is known to induce or accelerate hatching in amphibians and fish (DiMichele & Taylor 1980; Petranka et al 1982; Bradford & Seymour 1988), and may be the stimulus for early hatching of flooded eggs. It could also play a role in fungus-induced hatching. I cannot rule out a role for chemical or visual cues in fungus, wasp, or snake-induced hatching. None the less I have seen nothing to suggest that visual cues alone ever induce hatching, or that chemical cues alone can induce synchronous mass hatching.

### Embryonic Antipredator Defences

Until recently, embryos were not considered to have behavioural antipredator defences. Embryos are clearly unable to hide themselves from egg predators. They may struggle to free themselves from a predator, but with many predators this will be futile. However, they are not completely defenceless. Some embryos flee from predators by hatching, and this is an effective defence.

At least four species of anurans hatch early in response to egg predators (Warkentin 1995; Vonesh 2000; D. P. Chivers, personal communication). Furthermore, *A. callidryas* embryos hatch in response to multiple egg-stage risks, but the hatching pattern in each case is different. The differences between snake- and wasp-induced hatching appear adaptive given the different risks posed by each predator.

Behavioural antipredator defences of embryos are clearly not restricted to a single prey species, nor to a single type of predator, and have proven tractable for field and laboratory studies (Sih & Moore 1993; Warkentin 1995; Moore et al 1996). There are two major issues regarding embryonic antipredator behaviour that are worthy of further study. (1) What is its distribution in relation to phylogeny, ecology and life history? This information is necessary to begin to understand the evolution of the behaviour. (2) What is the mechanistic basis of the behaviour, and how does this vary among prey species, or for different predators on the same prey? Information on predator cues, the sensory physiology of embryos, and the mechanism of hatching will elucidate how embryos are able to recognize predators and respond by altering hatching. It may also reveal constraints on the expression of this behaviour. Integration of mechanisms,

ecology and evolution should yield fascinating insights on this important, relatively unstudied phenomenon.

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### References

- Boppré, M.** 1990. Lepidoptera and pyrrolizidine alkaloids: exemplification of complexity in chemical ecology. *Journal of Chemical Ecology*, **16**, 165–185.
- Bradford, D. F. & Seymour, R. S.** 1988. Influence of environmental PO<sub>2</sub> on embryonic oxygen consumption, rate of development and hatching in the frog *Pseudophryne bibroni*. *Physiological Zoology*, **61**, 475–482.
- DiMichele, L. & Taylor, M. H.** 1980. The environmental control of hatching in *Fundulus heteroclitus*. *Journal of Experimental Zoology*, **214**, 181–187.
- Dyer, L. A.** 1995. Tasty generalists and nasty specialists? Anti-predator mechanisms in tropical lepidopteran larvae. *Ecology*, **76**, 1483–1496.
- Gosner, K. L.** 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**, 183–190.
- Hayes, M. P.** 1983. A technique for partitioning hatching and mortality estimates in leaf-breeding frogs. *Herpetological Review*, **14**, 115–116.
- Jeanne, R. L.** 1991. The swarm-founding Polistinae. In: *The Social Biology of Wasps* (Ed. by K. G. Ross & R. W. Matthews), pp. 191–231. Ithaca, New York: Cornell University Press.
- Jeanne, R. L., Hunt, J. H. & Keeping, M. G.** 1995. Foraging in social wasps: *Agelaia* lacks recruitment to food (Hymenoptera: Vespidae). *Journal of the Kansas Entomological Society*, **68**, 279–289.
- Lacey, L. A.** 1979. Predação em girinos por uma vespa e outras associações de insetos com ninhos de duas espécies de rãs da Amazônia. *Acta Amazonica*, **9**, 755–762.
- McDiarmid, R. W.** 1978. Evolution of parental care in frogs. In: *The Development of Behavior: Comparative and Evolutionary Aspects* (Ed. by G. M. Burghardt & M. Bekoff), pp. 127–147. New York: Garland.
- Moore, R. D., Newton, B. & Sih, A.** 1996. Delayed hatching as a response of streamside salamander eggs to chemical cues from predatory sunfish. *Oikos*, **77**, 331–335.
- O'Donnell, S.** 1995. Necrophagy by Neotropical swarm-founding wasps (Hymenoptera: Vespidae: Epiponini). *Biotropica*, **27**, 133–136.
- Orians, G. H. & Janzen, D. H.** 1974. Why are embryos so tasty? *American Naturalist*, **108**, 581–592.
- Petranka, J. W., Just, J. J. & Crawford, E. C.** 1982. Hatching of amphibian embryos: the physiological trigger. *Science*, **217**, 257–259.
- Raveret Richter, M.** 2000. Social wasp (Hymenoptera: Vespidae) foraging behavior. *Annual Review of Entomology*, **45**, 121–150.
- Raveret Richter, M. A. & Jeanne, R. L.** 1985. Predatory behavior of *Polybia sericea* (Oliver), a tropical social wasp (Hymenoptera: Vespidae). *Behavioral Ecology and Sociobiology*, **16**, 165–170.
- Raveret Richter, M. & Jeanne, R. L.** 1991. Hunting behaviour, prey capture and ant avoidance in the tropical social wasp *Polybia sericea* (Hymenoptera: Vespidae). *Insectes Sociaux*, **38**, 139–147.
- Richards, O.** 1978. *The Social Wasps of the Americas Excluding the Vespinae*. London: British Museum (Natural History).
- Ruiz-Carranza, P. M. & Lynch, J. D.** 1991. Ranas Centrolenidae de Columbia I: propuesta de una nueva clasificación genérica. *Lozania*, **57**, 1–30.
- Sih, A. & Moore, R. D.** 1993. Delayed hatching of salamander eggs in response to enhanced larval predation risk. *American Naturalist*, **142**, 947–960.
- Villa, J.** 1979. Two fungi lethal to frog eggs in Central America. *Copeia*, **1979**, 650–655.
- Villa, J. & Townsend, D. S.** 1983. Viable frog eggs eaten by phorid fly larvae. *Journal of Herpetology*, **17**, 278–281.
- Vonesh, J. R.** 2000. Dipteran predation on the eggs of four *Hyperolius* frog species in western Uganda. *Copeia*, **2000**, 560–566.
- Warkentin, K. M.** 1995. Adaptive plasticity in hatching age: a response to predation risk trade-offs. *Proceedings of the National Academy of Sciences*, **92**, 3507–3510.
- Warkentin, K. M.** 1999a. Effects of hatching age on development and hatchling morphology in the red-eyed treefrog, *Agalychnis callidryas*. *Biological Journal of the Linnean Society*, **68**, 443–470.
- Warkentin, K. M.** 1999b. The development of behavioral defenses: a mechanistic analysis of vulnerability in red-eyed tree frog hatchlings. *Behavioral Ecology*, **10**, 251–262.
- Werner, E. E.** 1986. Amphibian metamorphosis: growth rate, predation risk and the optimal size at transformation. *American Naturalist*, **128**, 319–341.
- Werner, E. E.** 1988. Size, scaling, and the evolution of complex life cycles. In: *Size-structured Populations* (Ed. by B. Ebenman & L. Persson), pp. 60–81. Berlin: Springer-Verlag.
- Werner, E. E. & Gilliam, J. F.** 1984. The ontogenetic niche and species interactions in size structured populations. *Annual Review of Ecology and Systematics*, **15**, 393–425.