

Environmental and Developmental Effects on External Gill Loss in the Red-Eyed Tree Frog, *Agalychnis callidryas*

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ABSTRACT

I examined the effects of development, hatching, and oxygen availability on external gill loss in red-eyed tree frogs, *Agalychnis callidryas*. Under natural conditions, the arboreal embryos maintained large external gills until hatching, which occurred from 5–8 d after oviposition. At hatching, when tadpoles entered the water, external gills began to regress. In older hatchlings this process was extremely rapid. Gill circulation was lost on average within 16 min and sometimes within 5 min. Gills often regressed completely in under 2 h. Younger hatchlings reduced gill circulation, shortened and adducted their gills, then resumed normal circulation for some time after hatching; half had completely lost external gills within 24 h. Experimentally increasing the area of egg surface exposed to the air induced loss of external gills in unhatched embryos. Older hatchlings in hypoxic water without access to air maintained their external gills. This suggests that loss of external gills is a response to increased oxygen availability, rather than a response to hatching per se. Extended maintenance of external gills by large, late-hatching embryos may facilitate continued rapid development in closely packed eggs.

Introduction

Gills are a defining feature of amphibian larvae. The long-lasting external gills of salamanders and internal gills of anurans are physiologically important and well studied (reviewed in Burggren and Just 1992). In contrast, the external gills of anurans, ostensibly embryonic structures that are lost soon after hatching, are poorly studied, and their role in respiration is considered equivocal (Burggren and Just 1992).

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External gills in many anurans are small, simple structures. Some are unperfused before hatching (Duellman and Trueb 1986; Bradford 1990) and thus cannot contribute to embryonic respiration. Because anuran hatchlings are mostly small, simple diffusion may meet their respiratory needs (Burggren 1984). In support of this, *Xenopus* and *Rana* embryos and young larvae lacking functional hemoglobin show apparently normal rates of resting metabolism and development (Flores and Frieden 1969; Territo and Burggren 1998). Nonetheless, other anurans develop large, morphologically elaborate external gills (e.g., *Agalychnis callidryas*, Pyburn 1963; Warkentin 1999b; *Gastrotheca*, del Pino and Escobar 1981; *Hyla rosenbergi*, Kluge 1981). It seems unlikely that such apparently costly structures would have evolved, or been maintained, in the absence of physiological function.

The differentiation and regression of external gills are used as standard markers of development in staging tables for anurans, that is, these are viewed as canalized events (e.g., Nieuwkoop and Faber 1956; Gosner 1960). In some species, however, gill regression can be retarded relative to other development events, and the extent of this delay varies with environmental conditions (e.g., *Stephopaedes anotis*, Channing 1993; *Chiro-mantis xerampelina*, Seymour and Loveridge 1994; *A. callidryas*, Warkentin 1999b). Environmental regulation of gill-loss timing, like morphological complexity, suggests physiological function. Oxygen availability can affect the development of anuran external gills (Løvtrup and Pigon 1968), but no detailed study has addressed how the loss of these structures is regulated.

Embryonic respiration depends on diffusion through the egg capsule, and for some amphibian embryos oxygen availability limits metabolic rate (Seymour et al. 1991; Seymour and Roberts 1995). This is particularly likely to occur in large, warm eggs and eggs with reduced surface area for diffusion, especially late in development when metabolic rates of embryos are highest (Seymour and Bradford 1995). However, embryonic respiration can be limited by oxygen diffusion even in fully exposed, terrestrial eggs at 17°C (e.g., *Pseudophryne bibroni*, Seymour et al. 1991). After hatching, the egg capsule is no longer a diffusion barrier. Oxygen consumption sometimes increases immediately upon hatching, perhaps reflecting a release from diffusion constraints (Bradford and Seymour 1985; Burggren et al. 1990).

I studied external gill regression in a frog with large, warm, closely packed eggs, elaborate gills, and variable timing of gill loss. The red-eyed tree frog, *A. callidryas*, occurs in low-altitude tropical wet forests from the Yucatan through Panama. They

breed throughout the rainy season (late May–October in Costa Rica), attaching their egg clutches to vegetation overhanging ponds, and the tadpoles fall into the water at hatching. In Costa Rica, embryos hatch as early as 5 d after laying to escape from egg-eating snakes, but undisturbed embryos hatch later, usually at 7 or 8 d (Warkentin 1995). Late-hatched tadpoles are more developed and better able to escape from aquatic predators, compared to younger hatchlings, so the environmentally cued variation in hatching timing enhances survival (Warkentin 1995, 1999a, 1999b). *Agalychnis callidryas* eggs are moderately large (2.25-mm diameter before cleavage, Pyburn 1963), exposed to high ambient temperatures (26°C), and can reach a relatively advanced stage and large size before hatching (10–12-mm total length, Warkentin 1999b). Clutch size averages 40 eggs (± 2 SE, range 2–93, $N = 114$; K. M. Warkentin, unpublished data). Typical clutch dimensions are 2–3 cm by 3–4 cm (Pyburn 1963; K. M. Warkentin, personal observation). Hatchable eggs (5 d) are not separated by jelly (Fig. 1A; at 4 d, jelly thickness = 0.3 mm, Pyburn 1963). The embryos grow unusually large, elaborate external gills with a branched, filamentous structure (Fig. 1; drawing in Pyburn 1963; scanning electron micrographs in Warkentin 1999b). The gills are unpigmented, making circulation readily visible, and their regular structure facilitates recognition of unperfused vessels. Here, I examine the process and timing of external gill loss and its association with hatching, the development of other respiratory organs, and oxygen availability in the arboreal egg and the water.

I conducted three experiments. First, to document natural variation in gill loss and assess effects of development on the process, I compared gill regression in tadpoles induced to hatch at different ages. I then hypothesized that oxygen availability increases at hatching and that gill loss is a plastic response to such a change. I tested this in two ways: (1) I experimentally increased the surface area through which oxygen could diffuse into the egg and compared gill regression with control embryos, and (2) I compared gill regression of tadpoles hatched into hypoxic versus normoxic water and denied access to air.

Material and Methods

This research was conducted at Sirena Biological Station, Corcovado National Park, Costa Rica. All experiments used *Agalychnis callidryas* eggs collected from natural breeding sites within 2 d of oviposition that were then maintained over water in an open-air laboratory and misted daily with stream water to maintain hydration.

The section of anuran staging tables that applies to *A. callidryas* aged 5–8 d is based on external gill regression, thus these stages are not useful as an overall measure of development. Embryonic development is, however, very uniform so that within populations the age of embryos is predictable from their morphology and vice versa (Warkentin 1995, 1999b). Here, I

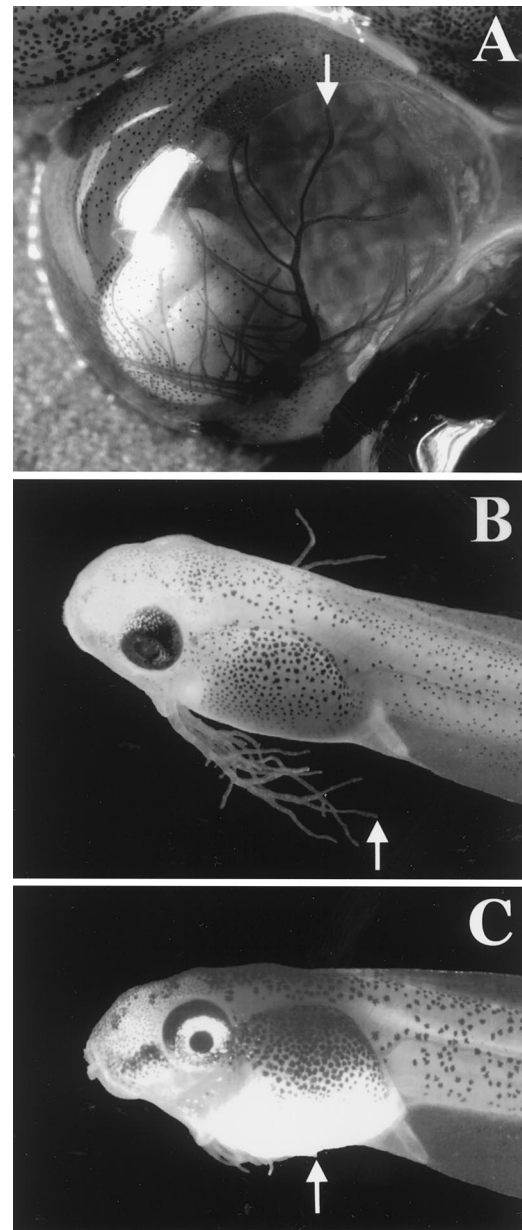


Figure 1. Changes in external gills following hatching in a 7-d *Agalychnis callidryas*. Just before hatching (A) gill filaments are spread out, positioned close to the vitelline membrane (right gills abducted), and fully perfused. Note the lack of jelly separating embryos. At 1 min after hatching (B), the gills are still long and perfused. At 3 min after hatching (C), the gills are much shortened, fully adducted, and have little circulation. The distal gill tip is marked at each time (arrow).

used age to characterize embryonic development; for morphological descriptions of Corcovado embryos at different ages see Warkentin (1999b). For convenience, I measured age from midnight on the night of oviposition; eggs are laid at night, mostly after 2200 hours.

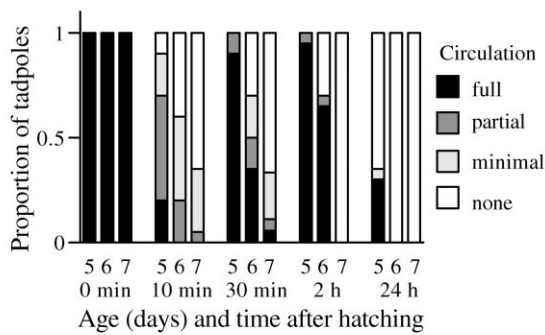


Figure 2. Changes in perfusion of external gills following hatching in *Agalychnis callidryas* hatched at different ages. Data are proportion of observed tadpoles showing each level of circulation at each time interval. Full = circulation through full length of all branchial vessels, corpuscles traveling in parallel in branches. Partial = some unperfused areas, or corpuscles traveling single file in branch vessels, but more than minimal circulation. Minimal = no more than two branch filaments perfused, trunk vessels narrowed overall or unperfused distally. $N = 20$ tadpoles per age, except for 7-d tadpoles at 30 min, $N = 18$ and at 2 h, $N = 16$.

Age Effects on Posthatching Changes in External Gills

I observed changes in the external gills of *A. callidryas* following hatching in animals hatched at 5, 6, and 7 d. I placed individual eggs in 5 mL of stream water in a 10-mL petri dish and observed them under a dissecting microscope at $\times 30$. Hatching occurred rapidly after submergence, as it does when hatchable eggs fall into ponds or are flooded by rising water (K. M. Warkentin, unpublished data). I recorded the length of external gills and their level of perfusion at least every 5 min for 30 min, then every 30 min until 2 h after hatching, and again at 24 h after hatching. I repeated this for two tadpoles hatched at each age from each of 10 clutches for a total of 60 hatchlings.

The regular structure of *A. callidryas* external gills, consisting of a gill trunk with one row of simple branch filaments extending from it (Pyburn 1963; Warkentin 1999b), allowed me to define easily distinguishable levels of perfusion. External gill perfusion was categorized and ranked for statistical tests as follows: (0) none = no movement of blood in external gills; (1) minimal = some circulation in main vascular trunk and two or fewer branches; (2) partial = circulation in more than two branches, but missing in some branches or branch sections, or limited to corpuscles traveling single file in branch vessels; (3) full = good circulation throughout the entire vascular field, with corpuscles traveling in parallel in branch vessels.

The external gills emerge immediately anterior to the yolk sac and extend caudally beside it. I, thus, estimated gill length (operculum to tip of terminal branch filament) relative to the length of the yolk sac in increments of one-eighth yolk length ($= 0.24$ mm). This method facilitates rapid measurement of

gill length in active tadpoles with minimal manipulation. I used average yolk sac length from 207 hatchlings to convert gill lengths to millimeters (Warkentin 1998).

Effect of Exposed Egg Surface

To alter the respiratory environment of embryos, I increased the area of egg surface exposed to air. I removed five eggs each from 10 clutches at age 3 d (stage 19, Gosner 1960; external gill circulation begins at stage 20). I suspended each egg in 3-mm diameter hexagonal nylon netting so that, except for the strip occluded by the encircling nylon filament, the egg surface was completely exposed. The remainder of each clutch (22 ± 2.4 eggs, mean \pm SE throughout) was left attached to its leaf, with the eggs in their natural (clumped) configuration. Interior eggs were about 25% exposed, and peripheral eggs were up to 50% exposed. All separated and clumped eggs were hung over water and misted daily; they remained well hydrated. A few netting cells adjacent to eggs held water droplets, reducing exposed egg surface by about 8% per water-filled cell. For analysis, embryos adjacent to one water-filled cell were combined with fully exposed eggs (high exposure) and contrasted with eggs in natural clumps (low exposure). Embryos adjacent to more than one water-filled cell (intermediate exposure) were treated separately. At 7 d, I examined all separated embryos that remained unhatched and two or three embryos from each clump of eggs at $\times 30$ under a dissecting microscope ($N = 24$ separated, 29 clumped embryos). Gill length and circulation were assessed as above for embryos still in the egg. For statistical comparisons, I used sibship averages for separated and clumped embryos ($N = 10$ sibships).

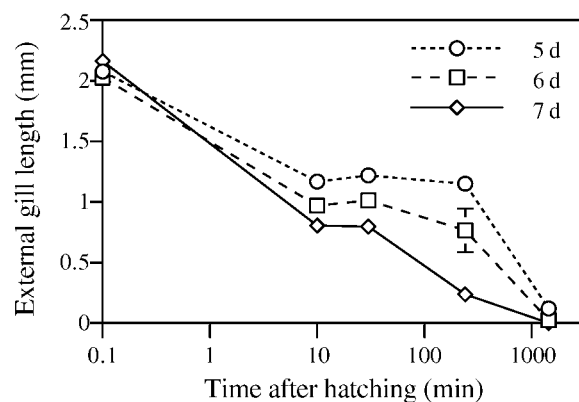


Figure 3. Changes in external gill length following hatching in *Agalychnis callidryas* hatched at different ages. Data are mean external gill length and SE. $N = 20$ tadpoles per age, except for 6-d tadpoles at 30 min, $N = 18$, and for 7-d tadpoles at hatching, $N = 16$ and at 30 min, $N = 18$. Some SE are smaller than the data points.

Table 1: Changes in external gill perfusion in *Agalychnis callidryas* tadpoles following hatching, for tadpoles hatched at three different ages

	Hatched at 5 d	Hatched at 6 d	Hatched at 7 d
Perfusion reduced	3.68 ± 1.68 (20)	4.58 ± 2.42 (20)	3.85 ± 2.22 (20)
Branches not perfused	5.72 ± 1.77 (6/19)	6.67 ± 2.38 (15/17)	5.93 ± 1.87 (17)
Basal trunk perfusion only	9.22 ± 1.45 (3/17)	11.02 ± 3.45 (14/15)	5.92 ± 1.77 (18)
No perfusion	9.68 ± .22 (2/20) ^a	16.92 ± 3.08 (9/20) ^a	16.52 ± 3.53 (17)
Increased perfusion	14.67 ± .90 (20)	19.92 ± 1.90 (14/20)	12.93 ± 1.02 (4/20) ^a

Note. Data are the average time (min) after hatching when conditions were first observed, expressed as mean ± SE (*N*). Due to tadpole activity and position, it was not possible to measure all variables for all animals, thus some sample sizes are less than 20. Not all measurable tadpoles showed all conditions; fractional sample sizes represent number of tadpoles showing each condition over number of tadpoles observed.

^a Brief, transient loss or gain of perfusion; see text.

Effect of Hypoxic Water

To alter the respiratory environment of hatchlings, I restricted access to air and manipulated dissolved oxygen. I placed sibling pairs of 7-d eggs into 10-mL petri dishes full of hypoxic and normoxic water and capped the dishes to prevent access to air (*N* = 20 clutches). Tadpoles hatched immediately, and I monitored gill length and circulation at ×30 as above for 20 min after hatching, alternating observations of experimental and control hatchlings within pairs (6.6 ± 0.3 observations each). Following experiments, tadpoles were transferred to normoxic water and allowed access to air; there was no mortality. To produce hypoxic water (24.6° ± 0.2°C and 2.4 ± 0.17 mg/L O₂), I boiled stream water and then cooled it in a sealed container. Unmanipulated stream water served as the control (24.2° ± 0.3°C and 7.4 ± 0.04 mg/L O₂). I measured oxygen level and temperature immediately before experiments in stock containers of water with a Nester Instruments model 8500 portable oxygen meter; I did not measure the passive rise in oxygen level in hypoxic water over the experiment. To calculate average gill length at standardized times for data presentation (Fig. 5), I used linear interpolation between adjacent data points when necessary.

Results

Changes in Gills after Hatching and Age Effects

Immediately before hatching, all 60 *Agalychnis callidryas* had rapid circulation in unconstricted blood vessels throughout their external gills. Within a few minutes of hatching, all had reduced the level of circulation (Figs. 1, 2). Reduced perfusion was visible in 58 tadpoles by the second observation (3.83 ± 0.25 min posthatching) and in the remaining two by the third observation (11.25 and 8.17 min). Initially, the lumen diameter of trunk vessels decreased and circulation in branch vessels stopped, usually with distal branches losing circulation before proximal ones. Eventually, all perfusion of the external gills stopped, typically leaving them colorless (Fig. 1C). As circulation in the gills decreased, the gills shortened and were ad-

ducted to lie flat against the body. In some animals, the post-hatching gill shortening was so rapid that I was unable to measure initial gill length, thus reducing sample sizes. Indeed, in a few animals, the drop in perfusion and shortening and adduction of gills began as the embryo was attempting to break through the vitelline membrane, moments before exiting the egg. Many tadpoles filled their lungs with air shortly after hatching. Even with air-filled lungs, 5-d hatchlings sank to the bottom, but 6- and 7-d hatchlings sometimes floated head up. In some cases, this made the extent of gill perfusion difficult to see, which is reflected in lower sample sizes (Table 1).

The process of external gill loss varied with age. Older hatchlings lost gill circulation and regressed external gills more rapidly than younger hatchlings (Page tests of ordered alternatives, Siegel and Castellan 1988: both *P* < 0.001; Figs. 2, 3; Table 1). In most 7-d hatchlings, gill circulation decreased continuously to cessation. A few individuals showed brief, transient increases in circulation, but all completely lost circulation within 2 h (Fig. 2). Similarly, gill shortening was continuous except for brief, transient increases in gill length in two tadpoles. Some had no visible external gills by 2 h after hatching, and none had external gills at 24 h after hatching (Fig. 3). Tadpoles hatched at 5 d first reduced gill perfusion, shortened and adducted their gills, then increased perfusion of their short, repositioned gills (Fig. 2). In some cases, gills lengthened slightly with the increased perfusion, but they remained adducted. All 5-d hatchlings had gill circulation after 2 h, and some still had very short, perfused external gills 24 h after hatching. Tadpoles hatched at 6 d showed both of these gill-loss patterns and intermediate variations, except that by 24 h posthatching, none had gill circulation, and only two had any visible external gill filaments (Figs. 2, 3).

Effect of Egg Surface Area Exposed to Air

Amount of egg surface exposed to air affected both external gill length and circulation (Wilcoxon tests: circulation, *P* = 0.002; length, *P* = 0.005). At 6 d, all embryos had perfused

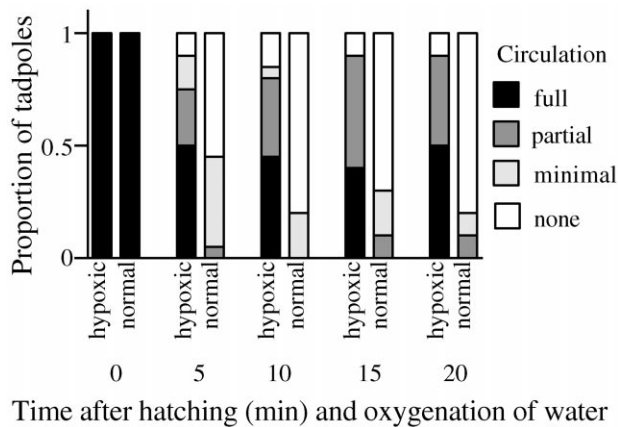


Figure 4. Changes in perfusion of external gills following hatching in 7-d *Agalychnis callidryas* hatched into hypoxic or normoxic water, without access to air. Data are proportion of tadpoles showing each level of blood circulation in their external gills at each time. See Figure 2 for definitions of circulation levels. $N = 20$ tadpoles per treatment.

external gills. By 7 d, no embryo in a high-exposure egg had any circulation in its external gills. In contrast, all low-exposure embryos had complete perfusion of the external gills. There was no overlap in gill size between treatments. The external gills of 7-d embryos in high-exposure eggs averaged 0.2 ± 0.04 mm long ($N = 21$ embryos). Three had no external gills at all, and the longest gills were only 0.6 mm. Gills of 7-d embryos in clumped eggs averaged 2.1 ± 0.06 mm long, with the shortest being 1.4 mm ($N = 29$). The external gills of three embryos in intermediate-exposure eggs were intermediate between those of embryos in high- and low-exposure eggs; the gills had partial circulation and were 1.0 ± 0.2 mm long.

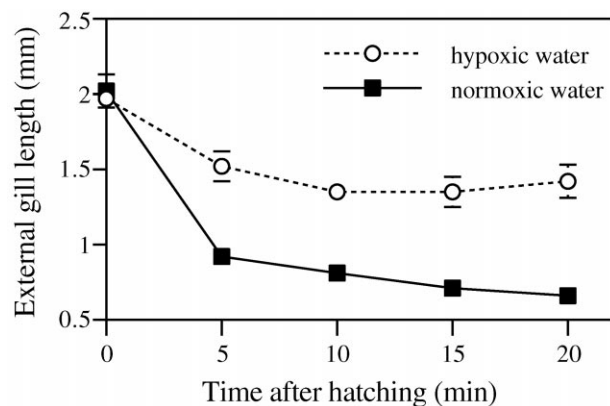


Figure 5. Changes in external gill length in 7-d *Agalychnis callidryas* hatched into hypoxic or normoxic water, without access to air. Data are mean and SE of gill length at each time. $N = 20$ tadpoles per treatment. Some SE are smaller than the data points.

Although I did not quantify development of nonbranchial structures, there were no obvious differences in mouthpart keratinization or intestinal development—both easily visible at $\times 30$ —between separated embryos and clumped embryos with some exposed surface. Occasionally, embryos were trapped behind their clutchmates without surface exposure. As in Seymour et al. (1995), these unexposed embryos were developmentally retarded, with differences apparent by the time exposed eggs reached stage 19 (Gosner 1960).

Effect of Hypoxic Water

All tadpoles hatched with fully perfused external gills, and gill length did not differ between hypoxic and normoxic water (Figs. 4, 5; Wilcoxon test: $P = 0.53$). Immediately after hatching in both treatments, gills shortened and their perfusion decreased (Wilcoxon tests: length, hypoxic, $P = 0.003$; normoxic, $P < 0.001$; perfusion, hypoxic, $P = 0.004$; normoxic, $P < 0.001$). In normoxic water, even without access to air, most tadpoles completely lost external gill circulation within 5–10 min (Fig. 4). The gills shortened to less than half their length at hatching within 5 min and continued to shorten (Fig. 5). The changes were, however, much smaller for tadpoles in hypoxic water. Within 5 min posthatching, gill length and level of perfusion differed between treatments, and they continued to differ (Figs. 4, 5; Wilcoxon tests: length, 5 min, $P < 0.001$; 10 min, $P = 0.003$; 15 min, $P = 0.001$; 20 min, $P < 0.001$; perfusion, all $P < 0.001$). Only three out of 20 tadpoles in hypoxic water completely lost external gill circulation, and one rapidly regained it. Over a third experienced no loss of circulation. The rest experienced partial or temporary loss of circulation. Gills also shortened less in hypoxic water, dropping to approximately 70% of length at hatching after 10 min and then lengthening slightly (Fig. 5).

Most tadpoles in hypoxic water performed rapid, deep buccal pumping, whereas those in normoxia pumped seldom and shallowly. Tadpoles in both treatments swam to the surface and butted against the lid, apparently seeking air. All tadpoles in normoxic water adducted their gills, while some tadpoles in hypoxic water abducted their gills.

Discussion

Rapid Loss of External Gills after Hatching

External gill regression in *Agalychnis callidryas* is rapid. Gills that are fully perfused and extend 60% of head-body length outside the operculum before hatching can be bloodless 5 min posthatching and absent within 2 h. Although comparably detailed data are not available for other anurans, external gill loss often appears to be more gradual (Table 2). Many aquatic-breeding frogs lose external gills sometime after hatching, and gill loss is not associated with a sudden environmental change. Both oxygen demand and oxygen uptake ability increase grad-

Table 2: Duration of the period of external gill regression in various anurans

Species	Time to Yolk Plug (Stage 12)	Duration of Gill Regression	Source
Rapid gill loss with sudden change of environment:			
<i>Agalychnis callidryas</i>	1.4 d	2–24 h ^a	This study
<i>Gastrotheca riobambae</i>	13 d	1 d ^b	del Pino and Escobar 1981
<i>Stephopaedes anotis</i>	<6 h ^c	Channing 1993
Gradual gill loss:			
<i>Xenopus laevis</i>	13.25 h	14–45 h	Nieuwkoop and Faber 1956
<i>Scaphiopus bombifrons</i>	8.75 h	6–12 h	Trowbridge 1942
<i>Heleioporus eyrei</i>	49.3 h	59.5–88.2 h	Packer 1966
<i>Bufo arenarum</i>	34 h (1.42 d)	1.5–3 d	Del Conte and Sirlin 1952
<i>Bufo valliceps</i>	10.5 h	9.5–20 h	Limbaugh and Volpe 1957
<i>Hyla avivoca</i>	10 h	31.5–47.3 h	Volpe et al. 1961
<i>Rana pipiens</i>	42 h	44–68 h	Shumway 1940
<i>Rana temporaria</i>	42 h (1.75 d)	0.5–2 d	Dabagyan and Sleptsova 1991
<i>Rana tigrina</i>	7 h	6–12 h	Agarwal and Niazi 1977

Note. Because developmental rates vary among species and at different temperatures, the time from fertilization to the yolk plug stage of gastrulation (stage 12, Gosner 1960) is provided for comparison with the duration of external gill regression. Except for *A. callidryas*, *G. riobambae*, and *S. anotis*, gill regression durations are based on staging tables. For durations from staging tables, minima represent the time from unilateral external gills (stage 24, Gosner 1960) to no external gills (stage 25), and maxima represent the time from opercular formation with bilateral gills remaining (stage 23) to no external gills. The actual duration of external gill regression falls between these values. Time is reported in hours or days, as in the original.

^a Depending on hatching age.

^b Gills retracted into the opercular chamber in 1 d; complete resorption of tissue in 1 wk.

^c In tadpoles that had facultatively delayed gill regression.

ually due to synthesis of metabolically active tissue and development of internal gills and lungs. Gradual loss of external gills in a stable environment is therefore not surprising. *Gastrotheca riobambae* is a clear exception to this pattern, with external gill regression occurring rapidly immediately after hatching (Table 2; del Pino and Escobar 1981). *Stephopaedes anotis* tadpoles transferred from hypoxic to well-oxygenated water also lose their external gills rapidly (Table 2; Channing 1993).

Agalychnis callidryas embryos progress steadily through the developmental stages described for other anurans until just before external gill regression at 5 d (stage 23, Gosner 1960). As measured by gill loss, embryonic development then stops; the duration of the last embryonic stage with bilateral external gills can be at least 6 d. Meanwhile, mouthparts, digestive organs, internal gills, and pigment cells differentiate, and tails and lungs grow (Warkentin 1999b). Under natural conditions, external gill regression begins at hatching, and its initiation is essentially decoupled from other developmental events. This suggests that external gills are important for embryonic respiration but unnecessary for hatched tadpoles and that their regression is a plastic response to improved gas exchange opportunities upon leaving the egg. The speed of external gill loss in *A. callidryas* and in *G. riobambae* (del Pino and Escobar 1981) is striking and may be an evolutionary result of the association of gill loss with hatching. Other anurans that lose

external gills in response to environmental change may also do so rapidly.

Development Affects External Gill Loss

Although the initiation of external gill regression is decoupled from other developmental events, the speed of the process varies. Older, more developed hatchlings lose their external gills more rapidly than younger hatchlings. With development, oxygen consumption must increase as metabolically active tissue is synthesized from relatively inert yolk. However, alternative gas-exchange surfaces also increase. Embryos at 7 d have lungs over 60% longer than at 5 d, more structurally complex internal gills, and a relatively larger skin surface due to tail growth (Warkentin 1999b). This appears to more than compensate for increased oxygen demand, making external gills unnecessary for older hatchlings. For the youngest hatchlings, with less internal and cutaneous respiratory surface, posthatching retention of external gills could temporarily augment oxygen uptake. This may be particularly relevant during exercise. Behavioral data suggest that avoiding benthic predators requires more swimming for the negatively buoyant younger hatchlings than for neutrally buoyant older hatchlings (Warkentin 1999a, 1999b). Alternatively, young hatchlings may not be physiologically competent to rapidly resorb their gills. However, the rapid

shortening of external gills immediately posthatching at all ages and the temporary cessation of external gill perfusion in some 5-d hatchlings does not support the hypothesis of developmental incompetence.

Oxygen Affects External Gill Loss

Although external gill regression in *A. callidryas* naturally begins at hatching, manipulations of oxygen availability can induce gill loss in embryos or delay it in hatched tadpoles. Thus, gill regression depends more on changes in the respiratory environment than on hatching per se. External gill loss by embryos in fully exposed eggs is probably a response to increased perivitelline Po_2 due to increased capsular conductance (Seymour and Bradford 1995). Gill regression also occurs in clumped embryos held in 42% O_2 /58% N_2 (K. M. Warkentin, unpublished data). The cessation of external gill perfusion and low rate of internal gill ventilation by 7-d hatchlings submerged in normoxic water suggests that cutaneous respiration may supply sufficient oxygen after hatching, at least for inactive animals. In contrast, 7-d hatchlings in hypoxic water maintain external gill perfusion. They also buccal pump rapidly, a common response of tadpoles submerged in hypoxic water (Feder 1983; Feder and Wassersug 1984).

Although less important than oxygen, hatching may also have a direct role in gill loss. This is supported by the initial drop in gill perfusion and gill shortening by tadpoles hatched into hypoxic water and by the fact that some animals started reducing perfusion and shortening and adducting their gills during the hatching process, before exiting the egg.

Since external gills respond to oxygen manipulations, their condition may indicate oxygen availability. If so, rapid gill loss at hatching suggests that hatching releases a respiratory constraint. Growth rates of *A. callidryas* also suggest a constraint on embryonic metabolism; growth and differentiation increase immediately upon hatching, whenever hatching occurs and well before feeding begins (Warkentin 1999b). Late-stage embryos in the similar-sized terrestrial eggs of *Pseudophryne bibroni* show oxygen limitation at 17°C, even with a fully exposed egg surface (Seymour et al. 1991); thus, oxygen limitation of warmer, clumped *A. callidryas* embryos seems reasonable.

External gills could facilitate oxygen uptake in two ways. First, the gills provide additional respiratory surface. Second, that surface is held close to the vitelline membrane, where oxygen enters the egg and Po_2 should be highest. Furthermore, the dense ciliation of external gills (Warkentin 1999b) should reduce any low-oxygen boundary layer around them.

The Role of External Gill Regression in Hatching Plasticity

Plasticity in the timing of hatching clearly enhances survival in *A. callidryas*. Late hatching allows vulnerable, young animals to avoid aquatic predators, and facultative early hatching allows

embryos to escape from attacks by egg-eating snakes and wasps (Warkentin 1995, 2000). Plastic timing of external gill regression may be an important component of adaptive plasticity in hatching. Specifically, delayed gill loss may facilitate delayed hatching, and rapid gill loss may be advantageous once in the water.

Oxygen stress induces hatching in amphibian and fish eggs (DiMichele and Taylor 1980; Petranka et al. 1982; Bradford and Seymour 1988). Enhanced oxygen uptake by external gills could extend the period before oxygen stress triggers hatching. Indeed, induced loss of embryonic external gills stimulates early hatching of *A. callidryas* eggs in air (K. M. Warkentin, unpublished data). External gill retention by embryos may also allow continued rapid development as oxygen demand increases. Delayed hatching without continued development is unlikely to reduce the posthatching risk from aquatic predators, although it can be advantageous in dry ponds (e.g., Bradford and Seymour 1985).

Rapid loss, or at least repositioning, of external gills after hatching may be equally important. External gills create drag, which decreases swimming performance, and large, abducted gills are particularly high in drag (Dudley et al. 1991). Thus, gill regression by hatched tadpoles may enhance swimming performance, which can be critical to survival with aquatic predators (e.g., Kaplan 1992; Parichy and Kaplan 1995; Watkins 1996).

This study demonstrates that external gill loss in *A. callidryas* is not a canalized developmental event but rather a plastic response to environmental change. Embryonic development can occur largely without oxygen transport in some amphibians and fish (Humphrey 1972; Smith and Armstrong 1993; Pelster and Burggren 1996; Territo and Burggren 1998), and in these cases specialized respiratory surfaces appear unnecessary. However, amphibians are notably diverse both ecologically and physiologically (Feder and Burggren 1992). Other species, under different environmental conditions, may benefit from specialized embryonic and larval respiratory structures. An adaptive role for the transient external gills of anurans seems most likely in species like *A. callidryas*, where these structures are both morphologically elaborate and regulated in response to environmental variation. Measurements of oxygen availability inside the egg and metabolic rates of gilled and gill-less animals of otherwise equivalent developmental stage would enhance our understanding of the physiological role of anuran external gills.

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