

## Notes on the Reproductive Biology of the Alabama Red Hills Salamander (*Phaeognathus hubrichti*)

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### INTRODUCTION

Living Amphibia exhibit two major life history modes, possession of an aquatic larval stage or direct development, with the latter assumed to be the derived evolutionary condition (Duellman and Trueb 1986, Wake 1989). A small group ( $n = 20$  species) of plethodontid salamanders, the subfamily Desmognathinae, is of great interest because its members display both developmental modes (Marks 1995). For decades the prevailing phylogenetic hypothesis for the group, based upon morphology and habitat, was a monophyletic sequence from the larger, more aquatic species that possessed the longest larval lives to two dwarf terrestrial species with direct development Dunn (1926). This "aquatic to terrestrial" hypothesis remained unchallenged even with the discovery of a new, giant, fossorial species, the Alabama Red Hills Salamander (*Phaeognathus hubrichti*), that was thought to be a third species with direct development (Highton 1961).

Recently, analysis of mtDNA sequences revealed that the terrestrial desmognathines form the three deepest branches in desmognathine phylogeny, compelling the authors to advance an alternative phylogenetic hypothesis that absence of an aquatic larval stage may be ancestral for desmognathines (Titus and Larsen 1996). Their hypothesis rested, however, on details of the developing embryo and hatchlings in the three species with direct development, but critical data on the eggs, hatchlings, and whether larvae exist in *P. hubrichti* are unavailable.

Aspects of the reproductive biology of the rare and secretive *Phaeognathus hubrichti* are difficult to observe in the field because the species is a burrower. One clutch laid by a female kept in captivity for six years apparently was unfertilized because the eggs failed to develop (Brandon and Moruska 1982). The large size and small number of ripe ovarian oocytes observed in preserved specimens, coupled with the unusual terrestrial burrowing behavior of the species, suggest the absence of an aquatic larval stage (Brandon 1965).

On several visits to one ravine in Butler Co., Alabama ( $31^{\circ}32'N$ ,  $86^{\circ}45'W$ ) during the spring and summer of 2002, I repeatedly observed a 105 mm SVL gravid female, discovered her eggs, kept them in captivity until they hatched, then returned the female and her clutch alive back into the field. Here I describe the field observations, eggs, embryos, and hatchlings.

### FIELD AND LABORATORY OBSERVATIONS

On 5-10-02 at 0015 hrs the head of a *P. hubrichti* was observed peering from a burrow on a  $55^{\circ}$  steep bank about 130 cm above the bed of the creek. She was teased out of her burrow by wriggling a twig in front of her, simulating a live food item. Yellowish spots showing through the skin of her abdomen indicated that she was carrying ripening ovarian ova (Figure 1). On 06-06-02 at 2300 hrs, the same gravid female with now larger ova was enticed out of the same burrow. She was replaced in her burrow at 2305 hrs but was observed peering out of the burrow again at 2335 hrs and continuously until 0100 hrs when I left. Neither headlights, photographic flashes, nor handling on two occasions caused her to retreat underground, so the likelihood that she might oviposit nearby seemed high.

On 06-29-02 at 2330 hrs I suspected that the gravid female might be brooding her eggs because she was not seen at the mouth of her burrow nor peering from other holes on the slope on which her burrow was located. Upon careful excavation of the bank, the female was located looped in angular cracks in the siltstone 30 cm straight back from the mouth of her burrow. She crawled into view revealing that she was still gravid and extremely plump with 9 large ova visible through her abdominal wall (Figure 1). She was returned to the cracks from which she had emerged and the excavation was carefully refilled with siltstone.

At 2345 hrs on 08-06-02, while re-excavating the cavity, a small chunk of siltstone fell into view with 6 large eggs dangling from one corner (Figure 2). Immediately the ceiling and back wall of the cavity were searched for the female but she was not seen. The eggs were 28 cm to the right of where she had been resting on 06-29-02 and about the same distance back from the surface of the bank. Following the disturbance created by my excavation 38 days previously, the female had simply moved a short distance laterally through the cracks in the siltstone and deposited her eggs. At 2350 hrs the female, now obviously spent, was located 20 cm deeper into the slope beyond where her eggs had lain and at the same horizontal level. No seepage nor trickling water was noted in the vicinity of the eggs and water was absent in the bed of the first order creek in whose ravine sidewall her burrow was located.

The eggs were transported to the laboratory where they were kept incubating in the dark at room temperature (75°F) in a plastic bag to retain humidity and suspended between chunks of moist siltstone taken from the excavation. On 08-14-02 the eggs appeared desiccated, so they were folded into saturated paper toweling where they quickly rehydrated. The clay, although moist, had a desiccating effect on the eggs which were touching the clay. The eggs were maintained on saturated paper toweling which was replaced every two days until the eggs hatched.

The mother was taken to the lab also, but kept apart from the egg clutch for three reasons. First, *Desmognathus* are known to ingest their eggs when the brooding female is disturbed (Fitzpatrick 1973). Second, there is a suspicion that female *P. hubrichti* may not brood her clutch (Brandon and Maruska 1982), so allowing the clutch to develop without the female present would give some evidence that brooding might not be necessary. And third, the female would be available so that the eggs could be rubbed over her skin if fungal or microbial activity was detected (Tilley 1972).

## RESULTS

The clutch consisted of only six eggs in spite of 9 ripening ova that had previously been visible through the female's body wall. Had 9 eggs been laid, three could have been ingested by the mother or by a potential predator, *Dicaelus dilatatus*, a large carabid beetle (Figure 3), three of which were found in the crevices in the clay within 20 cm of the eggs. Each egg hung from its own stalk, but all six were attached together on a larger pedicel (Figure 2). The slightly oval eggs measured no less than 9 mm in their least dimension by no more than 10 mm in their greatest.

When found on 08-06-02 the embryos were in an advanced stage of development with fully developed eyes, a long tail, four limbs, four digits on the manus, and five on the pes. The 6 embryos were at a stage comparable to at least stage 31 of *Desmognathus aeneus* (Marks and Collazo 1998). They lacked a dorsal pigmentary pattern of paired oval blotches such as is found in many of their relatives of the genus *Desmognathus* (Means 1974), but were evenly pigmented with dark brown melanophores on the head, dorsum, tail, limbs, and flanks. Only the large, round belly was unpigmented with yellow yolk showing.

A striking feature of the embryos was large, feathery gills that were easily visible extending over the large, yellow yolk sac when the eggs were first found on 08-06-02 (Figure 4). By 08-22-02 it was difficult to see the gills because the embryos were large and dark but two eggs accidentally broke open revealing red, bushy gills on one individual. The gills of the other had been broken off. The two individuals were placed together in a ceramic bowl with enough water to cover them, then sealed in a plastic bag to prevent desiccation. Subsequently, on 08-25-02, three more eggs hatched, and the last of the 6 eggs hatched the next day. All six had large, bushy gills consisting on the left side of 3 stout rami with up to 9 fimbriae each (Figure 5). On the right side four individuals had 3 rami but one had two (Figure 6) and another had only one stout ramus (Figure 7), all with numerous fimbriae.

The six hatchlings averaged 22.5 mm SVL (range = 21-24), 14.5 mm tail length (range = 13-15), and 36.8 mm total length (range = 35-38). Each had an average of 21 (range = 20-22) costal grooves between the axilla and groin. The mouth of hatchlings appeared externally to be fully formed in the morphology of the adult salamander and not possessing the labial folds of the suctorial mouths of larvae of *Desmognathus*. Tails of the hatchlings were round as in adults with no trace of fleshy tail fins. Hatchling eyes did not blink when touched, but vestiges of eyelids were present.

The first two hatchlings whose eggs broke open accidentally were placed in water to simulate the aqueous environment of their eggs. When the last individual hatched on 08-26-02, I placed it, and one that hatched on 08-25-02, on the moist substrate of crumbled siltstone from their natural habitat, leaving the other two pairs in water in a flat-bottomed bowl that was tipped slightly in order to present a choice of total immersion in water or a dry surface to crawl onto. Twenty-four hours later on 08-27-02, the two individuals on the natural substrate had lost one-half the volume of their gills (rami and fimbriae) while only a slight amount of reduction of the fimbriae was noted in the others. By 09-01-02, however, the gills of all 6 hatchlings were reduced about equally to stubs with no gill fimbriae. On 09-05-02, the gills of all 6 were completely gone and the hatchlings appeared completely terrestrial (Figure 8) in spite of the fact that 4 had unlimited access to water and had remained in water most of the time.

Oviposition probably took place later than 06-29-02 because that was the date when the gravid female was prematurely excavated from her subterranean habitat, but it must have taken place soon thereafter because of the advanced stage of development of her eggs on 08-06-02. The only other record of oviposition in *P. hubrichti* took place in a captive female that was kept in the dark for more than five years (Brandon and Maruska 1982). The female oviposited on 07-01-76 after her room was equipped with fluorescent lighting to match the Cincinnati photoperiod. These two instances indicate that oviposition takes place in late June and early July.

## DISCUSSION

As predicted by Brandon (1965), *Phaeognathus hubrichti* does not possess a free-living, aquatic larva. Hatchlings did possess fully developed, branched gills, but the gills disappeared rapidly in less than 10 days post-hatching whether the hatchlings were maintained immersed in water or on dry substrate of their habitat. Maximum incubation time, assuming the female laid the eggs on the night of 29 June 2002, was 58 days. An incubation period of 68-75 days has been documented for eggs of *Desmognathus aeneus*, a relative with direct development (Marks and Collazo 1998). Harrison (1967) estimated incubation time for *D. aeneus* as 34-45 days, but Marks and Collazo (1998) believed the discrepancy between the two estimates was due to differences in laboratory temperatures during development, resulting in premature hatching in Harrison's samples.

Premature hatching could result in individuals that had not resorbed their gills and lacked eyelids like the *P. hubrichti* hatchlings described here. The eggs described here were raised at a temperature (75°F) that was warmer than the incubation temperature of the natural environment. The ground temperature (> 10 cm) for an area is approximately equal to an area's annual average air temperature, which in the geographic range of *P. hubrichti* is about 68°F (National Oceanic and Atmospheric Administration 2001). It is likely that, under cooler temperatures and longer incubation times—and considering the rapidity with which these hatchlings lost their gills, labial folds, and developed eyelids (10 days), *P. hubrichti* hatchlings are completely metamorphosed under normal conditions.

The long-standing hypothesis that the smallest desmognathines (*D. aeneus*, *D. wrighti*) represent the end of a graded phylogenetic decrease in body size and a decrease in aquatic habitats (Dunn 1917, 1926; Wilder and Dunn 1920) was challenged by Titus and Larsen (1996) whose mitochondrial DNA analysis positioned these two species basally in the phylogenetic tree of the Desmognathinae. Their data also showed, however, that *Phaeognathus hubrichti*, the longest and most terrestrial of all desmognathines, represented the first phylogenetic branching event in the subfamily and was even more basal than the other two lineages. If the principle of parsimony is rigorously applied to hypotheses about the evolution of ecology, life history, and morphology in desmognathines, Titus and Larsen (1996) suggested that direct development is ancestral for the Desmognathinae since all three basal desmognathine lineages possess it.

On the other hand, convergence could explain how direct development could have evolved independently in one or all of the three unbranching basal lineages in very different habitats having similar strong selective pressures for terrestrial life. The two terrestrial species, *D. aeneus* and *D. wrighti*, have retained the standard body shape and 14 trunk vertebrae of all other desmognathines, but *P. hubrichti* has evolved an additional 7 vertebrae probably in exclusive response to living underground in the cracks and fissures in the Tallahatta and Hatchetigbee geological formations. These geological formations were deposited at the bottoms of deep Eocene seas (Laws and Thayer 1992) and weren't available for colonization by plethodontid salamanders until at least the early Miocene, 30 mya, long after the basal lineages had diverged from other desmognathines.

Elongate body shape is characteristic of fossorial vertebrates (Brown and Lasiewski 1972) and *P. hubrichti* possesses many fossorial morphological adaptations such as solidly constructed skull, small nostrils, modified eyelids, small legs, large body size, extra trunk vertebrae, and a prehensile tail (Valentine 1963, Blair 1967). Deep ravines in other and even adjacent geological formations are abundant in the Coastal Plain of the southeastern U. S. (Means 2000), but *P. hubrichti* is completely restricted to the Tallahatta and Hatchetigbee formations. It is so highly adapted to its special habitat and has such low vagility that populations east and west of the Sepulga River may have been isolated long enough to have diverged into separate species (McKnight et al. 1991). It is reasonable, therefore, that the fossorial adaptations possessed by *P. hubrichti* are specialized for the unique and very restricted habitat in which the species lives today and in which its ancestors probably have been confined since the Miocene. And it is also reasonable that direct development evolved in these same habitats as a specialization to facilitate fossorial morphology and behavior.

Whether maternal brooding behavior exists in the Red Hills Salamander, such as is present in species of Desmognathus and plethodontids in general, has not yet been demonstrated. Brandon and Maruska (1982) did not observe it in their captive female, but the eggs were infertile and soon decomposed. The female in this study was only 20 cm away from her presumed clutch, but she could have been frightened away during excavation. Her six eggs survived to hatching in the laboratory over a 20-day period without her physical contact, however. Considering that maternal brooding behavior would, at least, prevent predation on the eggs by other *Phaeognathus*, other syntopic plethodontids (*D. monticola*, *D. conanti*, *Pseudotriton ruber*, *Eurycea guttolineata*), carabid beetles, and other invertebrates, one would not expect this aspect of the biology of a burrowing species to have been

lost evolutionarily. And female *Phaeognathus* can deliver a hard bite, which I experienced several times while attempting to photograph the mother. The limited evidence seems to indicate the absence of brooding behavior in *P. hubrichti*, but I believe that the behavior of mature females toward their egg clutch requires more study.

A number of studies have mentioned the burrowing behavior of *P. hubrichti* (Brandon 1965, French and Mount 1978, Dodd 1991, Gunzburger and Guyer 1998). Physically tunneling into the dense Tallahatta claystones and siltstones, however, is impossible for a salamander because these materials are very dense and hard. I found it difficult to stab the tines of a potato rake or geological pick into them. While examining the habitat during the excavation of this clutch, I was impressed with the fact that only a few inches below the surface of the ground the claystone and siltstone matrix was comprised of small, polygonal blocks, loosely compacted, but often exhibiting interstitial spaces large enough for salamanders and invertebrates to roam freely in them. Friable soil forms from these fine-grained parent materials, but it is only a few inches deep, mantling the matrix below. *P. hubrichti* is capable of burrowing through the friable soil, but the main passageways it inhabits may be the natural cracks and fissures formed in the claystones and siltstones and not cavities that *P. hubrichti* actually excavates. Clays of the Hatchetigbee and Tallahatta formations in which *P. hubrichti* lives are diatomites and siliceous mudstones. These are moderately to extremely hard clays that typically display blocky, conchoidal, or sub-conchoidal fracturing (Laws and Thayer 1992).

The female of this study was present at her burrow mouth in early May and June, but not visible on 06-29-02 or 08-06-02. On both latter occasions she was 30 – 48 cm deep in the bank behind her burrow mouth, remaining there in spite of having been excavated on 06-29-02. In future surveys of live animals, I caution fieldworkers to be aware that adult females may not be present at ground surface during the season of oviposition (late June-early July) and possibly throughout the incubation period, July and August.

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