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Stanley E. Trauth
Arkansas State University

Robert L. Cox Jr.
Arkansas State University

J. D. Wilhide
Arkansas State University

Hilary J. Worley
Arkansas State University

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Egg Mass Characteristics of Terrestrial Morphs of the Mole Salamander, *Ambystoma talpoideum* (Caudata: Ambystomatidae), from Northeastern Arkansas and Clutch Comparisons with Other *Ambystoma* Species

Stanley E. Trauth, Robert L. Cox, Jr.¹, J.D. Wilhide and Hilary J. Worley
Department of Biological Sciences
Arkansas State University
State University, AR 72467-0599

Introduction

Aquatic eggs and/or egg masses of many salamander species normally increase in mass by acquiring a considerable amount of water via osmosis immediately following oviposition (see Duellman and Trueb, 1986). Most of this water gain is restricted to an area between the vitelline membrane which immediately surrounds the ovum and the outer egg capsule. Within this region there can be as many as eight concentric capsular rings per egg (Salthe, 1963); as these capsules expand, a gelatinous appearance is bestowed upon the single egg and/or egg masses. Not only do egg capsules serve to protect the developing embryo from predation and physical harm, but they also are vital to successful fertilization of the egg and must be present for hatching to occur (Duellman and Trueb, 1986).

There are six species of ambystomatid salamanders that occur in Arkansas (Conant and Collins, 1991); of these, five species (*Ambystoma annulatum*, *A. maculatum*, *A. talpoideum*, *A. texanum*, and *A. tigrinum*) normally lay their eggs by attaching them to submergent vegetation in the form of masses or clusters, whereas the other species (*A. opacum*) deposits its eggs in a terrestrial nest at the edge of a pond or pool of water. Among the former species, only the mole salamander (*A. talpoideum*) has been shown to vary geographically by exhibiting two egg-laying patterns (see review in Semlitsch and Walls, 1990). Within the Mississippi River Valley ecoregion this species deposits eggs in the form of egg clusters (Shoop, 1960; Raymond and Hardy, 1990), whereas in the Atlantic Coastal Plain single eggs are attached to vegetation (Semlitsch and Walls, 1990). In addition, this species is polymorphic (exhibition paedomorphic and metamorphic forms) in Arkansas (Trauth et al., 1993) as well as in other parts of its range (see Scott, 1993).

The present study documents egg mass and clutch characteristics of terrestrial morphs of *A. talpoideum* in Arkansas. In addition, we compare similar egg mass traits of this species with another ambystomatid species, the spotted salamander (*A. maculatum*). Lastly, we summarize egg mass and/or clutch size of *Ambystoma* species in Arkansas.

Materials and Methods

Egg masses of the terrestrial morph of the mole salamander, *Ambystoma talpoideum*, were collected from two temporary ponds in Greene County, Arkansas, in December of 1988 and 1994. These seasonal, temporary ponds (abandoned gravel pits) differed markedly from those permanent ponds that typically contain paedomorphic individuals. Egg clusters (with embryos mostly in early stages of development) were taken to the laboratory and placed into fixative (10% formalin) within 24 hr after collection. All egg masses are housed in the Arkansas State University herpetological collection.

Laboratory procedures included the recording of mass (g) and number of eggs per egg mass. While measuring egg masses, individual masses were removed from fixative and blotted dry; mass was recorded with a triple-beam balance (to the nearest 0.1 g). Egg masses of the spotted salamander (mostly collected on 8 March 1988 from permanent ponds in Independence, Izard and Marion counties) were processed in a similar manner. (In order to support the contention that preserved egg masses actually gain mass following preservation nine egg masses of *A. maculatum*, collected on 28 January 1995, were weighed (massed) prior to preservation and then one month thereafter. In all cases, these egg masses gained at least 100% in total mass.) Mean values in Table 1 are accompanied by \pm two standard errors.

Results and Discussion

The morphology of egg masses of metamorphic *A. talpoideum* is shown in Fig. 1. The globular nature of these egg masses supports the dichotomous pattern of egg mass structure for this species as discussed by Semlitsch and Walls (1990). The masses differed structurally from those of *A. maculatum* (illustrated by Gilbert, 1942) mainly by being more fragile. For example, upon collection in the wild, egg masses of *A. maculatum* are typically characterized by their turgidity or an ability to withstand manipulation. Egg masses of *A. talpoideum* also retain a globular appearance as viewed in recently-oviposited masses

¹ (RLC-deceased)

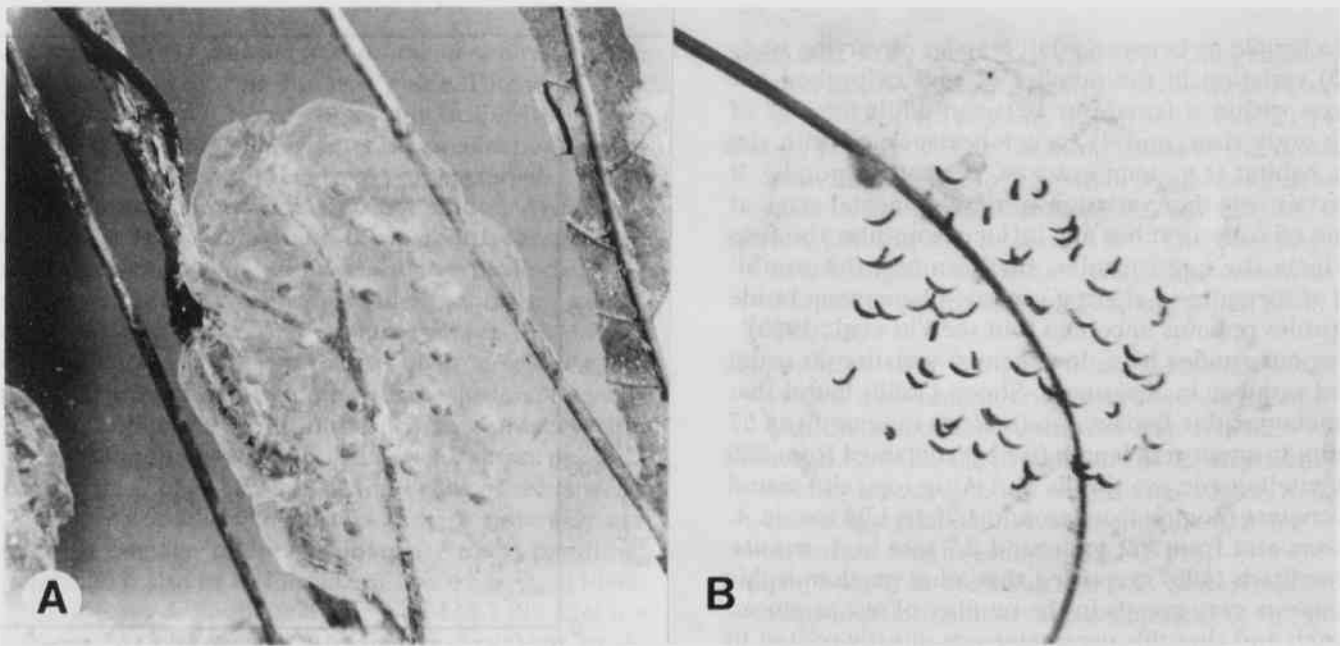


Fig. 1. Egg masses of the terrestrial morph of *Ambystoma talpoideum* collected in Greene County, Arkansas. A. Recently-oviposited egg mass (shown attached to a twig) with around 32 eggs. B. Egg mass with around 55 embryos nearing hatching.

(developmental stage determined by embryonic stage) as well as in older masses whose embryos are nearing hatching (Fig. 1 A and B, respectively). The gelatinous capsules of each egg mass eventually break down or weaken in both species as embryos near hatching (Fig. 1B). The nature of the egg mass of terrestrial morphs of this species also differs sharply from those of the paedomorphs (observed in Arkansas) whose eggs are laid singly (sometimes attached to one another) and are very fragile (S.E. Trauth, unpubl.).

Table 1. Data on the number of eggs per egg mass (or clutch size) for *Ambystoma* species documented in Arkansas.

Species	N	Average Egg Mass Size (\pm 2 SE)	Range	Source
<i>A. annulatum</i>	-	14.0	4-31	Trapp, 1956
	-	7.8	2-17	Trapp, 1956
	-	-	1-50+	Spotila and Beumer, 1970
<i>A. maculatum</i>	75	148.7 (\pm 14.9)	20-334	This study
<i>A. opacum</i>	10	107.1	87-124	Trauth et al., 1989b
<i>A. talpoideum</i>	60	41.1 (\pm 5.0)	12-99	This study
<i>A. texanum</i>	34	12.6 (\pm 1.5)	5-22	Trauth et al., 1990
<i>A. t. tigrinum</i>	2	130.5	96-165	Trauth et al., 1990

Figure 2 illustrates a comparison of mass gain in egg masses (following preservation) of *A. talpoideum* and *A. maculatum*. Although data shown in Fig. 2 are not intended to actually represent a measure of mass gain of egg masses in the wild (nor do data in Fig. 2 signify or express any inherent osmotic quality of these egg clusters), the degree of intraspecific variation associated with egg mass size and maximum mass gain is intriguing. If we assume that osmosis is not encumbered or halted after preservation (see above), then each egg mass of equal egg number and size should presumably undergo a similar mass gain. A comparison of the two December collections (collected six years apart) for *A. talpoideum* (Fig. 2) revealed differing mass gains per egg mass of equal egg number. This was also observed for egg masses of *A. maculatum*. These differences suggest individual variation in ovipositing females and that this variation presumably is grounded either in the selective pressure on females ovipositing in temporary ponds vs. permanent ponds or in the inherent variability of reproductive and/or life-history characteristics associated with ovipositing females in any given population [as has been observed in many ambystomatid salamanders (e.g., see Walls and Altig, 1986)]. For instance, differences in mass in egg masses of similar size could be the result of the following types of variation: 1) variation in ovum size at ovulation within a female or between adult females of varying body sizes, 2) variation in egg size (with egg capsules) at oviposition

within a female or between adult females of varying body sizes, 3) variation in the number of eggs oviposited per egg mass within a female or between adult females of varying body sizes, and 4) variation associated with the aquatic habitat (e.g., temporary vs. permanent ponds). It is uncertain whether variation in developmental stage at the time of collection has any influence on how the fixative affects the egg capsules; furthermore, the overall effects of formalin in altering osmotic phenomena inside egg capsules remains uncertain (but see Vitt et al., 1985).

Previous studies have documented variation in ovum size and number in *Ambystoma*. Shoop (1960) found that adult metamorphic female *A. talpoideum* ranging from 57 to 66 mm in snout-vent length ($n=14$) contained from 226 to 401 vitellogenic ova. Walls and Altig (op. cit.) found ova diameters ranging from around 1.76 to 1.93 mm in *A. talpoideum* and from 2.2 to around 2.7 mm in *A. maculatum*. Semlitsch (1987) reported that adult paedomorphic *A. talpoideum* vary greatly in the number of ova produced per clutch and that this parameter was directly related to female body size. Furthermore, whether a pond is temporary or permanent has been shown to affect the size as well as number of eggs per clutch in *A. maculatum* (Woodward, 1982). Woodward (op. cit.) found that larger females in permanent ponds produced larger eggs but do not increase the number of eggs per clutch and that permanent pond females produced more eggs per clutch than do temporary ponds females. Partitioning of clutches could, therefore, result in both large and small females ovipositing similarly-sized egg masses; however, each egg mass could conceivably gain a different amount of water, because of egg size differences.

Our data in Fig. 2 also suggests that maximum mass values for egg masses exhibited by both salamander species are consistent with differences in egg size. For example, in *A. talpoideum* an egg mass of 14 eggs had a maximum mass of 44.6 g, whereas an egg mass of 75 eggs had a maximum mass of 192.6 g. Therefore, the average mass per egg for the former is 3.2 g, and the average mass per egg for the latter is 2.6 g. On the other hand, in *A. maculatum* an egg mass of 120 eggs had a maximum mass of 214.4 g, whereas an egg mass size of 299 eggs had a maximum mass of 331.6 g. Therefore, the average mass per egg for the former is 1.8 g, and the average mass for the latter is 1.1 g. These ranges suggest that the ability to gain mass is more pronounced in the smaller, more fragile egg capsules of *A. talpoideum* than in the larger eggs with firmer egg capsules of *A. maculatum*.

A summary of egg mass size (or clutch size as in *A. opacum*) for *Ambystoma* species collected in Arkansas is shown in Table 1. Because five of the six species of *Ambystoma* are aquatic egg-layers and because interspecific overlap in egg mass size occurs among several species, the field identification of a particular species' egg mass in

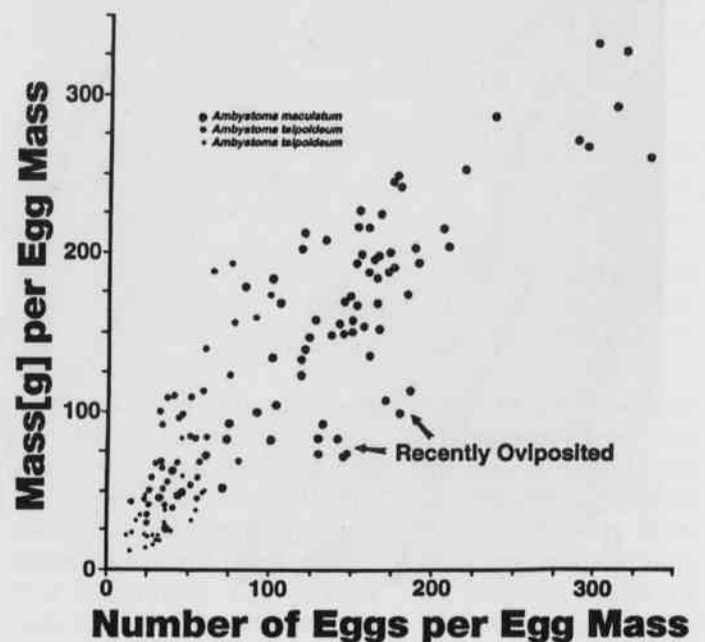


Fig. 2. Number of eggs per egg mass as a function of mass (g) per egg mass of preserved egg masses in *Ambystoma talpoideum* and *A. maculatum*. Clusters of recently-oviposited eggs (as shown by arrows) were not preserved prior to mass determination. Small solid circles = egg masses collected on 19 December 1988; intermediate-sized, solid circles = egg masses collected 19 December 1994.

geographic areas of sympatry can often be difficult, especially when several species are ovipositing in the same pond or ditch at the same time. Yet, there are species-specific differences in the annual timing of oviposition as well as preferred egg-laying habitats that can usually assist in assigning egg clusters to a species of *Ambystoma*. No reliable established suite of egg mass characteristics can easily separate species in some situations. In Arkansas, we suggest that egg masses with eggs numbering over 100 per mass (and oviposited in ponds) be considered to be those of either *A. maculatum* or *A. tigrinum*. Winter breeding of *A. annulatum* (not exclusively during the fall months; see Trauth et al., 1989a) combined with their similar egg mass size with *A. talpoideum* and *A. maculatum* can make distinguishing egg masses ranging from 25 to 100 eggs problematic. In this case, egg masses of *A. maculatum* can be separated from the other two based on the greater turgidity of the mass (in *A. maculatum* vs. *A. annulatum*) along with the possibility of the presence of a green algae in *A. maculatum* (Gilbert, 1942); separation of *A. talpoideum* from the other two would be based upon greater fragility of egg masses. In egg masses fewer than

25, as seen in *A. talpoideum* and *A. texanum*, a reliance on egg-laying habitat may be the best guide. Ditches and/or small temporary pools are commonly utilized by *A. texanum*, whereas *A. talpoideum* generally prefers ponds.

In summary, data on egg mass characteristics of the terrestrial morph of the mole salamander, *Ambystoma talpoideum*, were examined and compared to those of the spotted salamander (*Ambystoma maculatum*) collected elsewhere in Arkansas. We also provide a summary of egg mass traits for other *Ambystoma* species inhabiting Arkansas. Although the egg masses of *A. talpoideum* (terrestrial morph) are oviposited in fragile clusters, they can withstand collection, manipulation, and preservation while retaining their globular-like, structural morphology. By comparison, eggs laid by paedomorphs of this species are normally not found in clusters and are extremely susceptible to damage or loss of integrity upon handling. Average clutch size in *A. talpoideum* was 41.1 eggs (14-99; n = 60), whereas *A. maculatum* averaged 148.7 (20-334; n = 75). Mass (g) changes while in preservative in *A. talpoideum* and *A. maculatum* indicated that eggs of both species gained mass. The reliability of using preserved (museum) egg masses to provide information on selected reproductive characteristics in salamanders remains speculative and awaits further scrutiny.

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