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Variation and Phenology of *Ambystoma mavortium* (Western Tiger Salamander) Eggs in Colorado

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***Abstract*.—Western Tiger Salamanders (*Ambystoma mavortium*) occur throughout Colorado, occupying an elevational range in the state from about 1,045 m to 3,660 m. These salamanders exhibit great variation in the onset of egg deposition. For breeding localities at elevations below about 2,000 m with permanent water, egg deposition takes place as early as March. At temporary pools at low elevation, rainfall, which may occur as late in the activity season as August, initiates breeding and egg deposition. At sites about 2,000 m and above, eggs appear later in the season with increasing elevation. Eggs of *A. m. mavortium* at low elevation sites have comparatively reduced jelly layers, but the diameter of the jelly layers surrounding the ovum increases with elevation for *A. m. nebulosum*, *A. m. melanostictum*, and *A. m. mavortium* × *nebulosum* intergrades.**

Key Words.—Colorado; egg characteristics; embryo; mole salamanders; phenology

Introduction

Western Tiger Salamanders (*Ambystoma mavortium*) occur throughout Colorado and occupy an elevational range of about 1,045 m to 3,660 m (Hammerson 1999; Stephen Mackessy, unpubl. report). Studies of mitochondrial DNA in the tiger salamander complex distinguished an eastern lineage (*Ambystoma tigrinum*) from the western lineages, including *A. mavortium* and its subspecies (Shaffer and McKnight 1996). Reese (1969) mapped the distribution of three subspecies present in Colorado. *Ambystoma m. mavortium* primarily inhabits the low elevations of the eastern plains, whereas *A. m. nebulosum* occurs in mountainous areas in the western half of the state; some intergradation between *A. m. mavortium* and *A. m. nebulosum* occurs where the two subspecies meet. *Ambystoma m. melanostictum* occurs in north-central and northwestern Colorado with limited intergradation with *A. m. nebulosum* (Fig. 1).

The larval and adult life-history stages of *A. mavortium* span months to years. Although larvae at many breeding sites metamorphose by the end of summer, at other sites larval or paedomorphic individuals (salamanders retaining the larval form but sexually mature) are resident throughout the year (Hammerson 1999). The presence of eggs and embryos at a breeding site is comparatively transitory. In Colorado, Reese (1969) stated that eggs generally hatch in 12–14 d or longer at high elevations. Laboratory observations for *A. m. nebulosum* in Utah indicate that development to hatching lasts from 6.5 d to 2–3 weeks depending on temperature (Tanner et al. 1971). In addition, the eggs and embryos can be as small as 4.2 mm total diameter and easily overlooked (pers. obs.). Consequently, fewer observations of eggs and embryos make it into the scientific literature, reports, or citizen science projects. Here I use the terminology recommended by Altig and McDiarmid (2007, 2015), who considered eggs to includes both the gamete (ovum) and jelly layers produced in the oviduct. The egg diameter is the distance across the outer jelly layer for a particular egg. Hatching, the stage at which the larva emerges from the egg (Voss 1993), occurs at approximately Harrison stage 40 (pers. obs.). In this paper I summarize the variability in the appearance of *A. mavortium* eggs, their modes of deposition, and observed phenology in Colorado.

Methods

Generally incidental to other work, I observed eggs and embryos at *A. mavortium* breeding sites on 34 occasions between 1976 and 2020. When possible, I photographed representative eggs and made notes on the manner of deposition and other features. I noted where the eggs were deposited: on the substrate (pond or pool bottom); on submerged branches; or on vegetation. At 14 sites I measured an average of 10 (range one to 38) egg diameters (ovum/embryo plus jelly layers). At some sites I directly measured egg diameters with calipers, while at other sites I estimated egg diameters from photographs containing images of eggs and a ruler. I searched pertinent literature, reports, and obtained personal communications to compile additional information on egg phenology or characteristics (Hamilton 1949; Reese 1969; Sexton and Bizer 1978; Kiesecker 1991; Lambert 2001). Sources were excluded if they lacked the date of observation or enough information to determine elevation. I mapped localities using TOPO! 4.5.0 (National Geographic Holdings, San Francisco, California) to obtain estimates of breeding site elevations. Seasonal temperature and hydrologic regimes differ considerably between lowland sites versus sites in the mountains (considered here to be localities above 2,000 m elevation), so these were analyzed separately in terms of date of egg deposition.

Jelly layers of amphibian eggs rapidly absorb water after oviposition (Stebbins 1949; Thurow 1997), and I observed all eggs after this initial water absorption. Because I encountered eggs and embryos at different

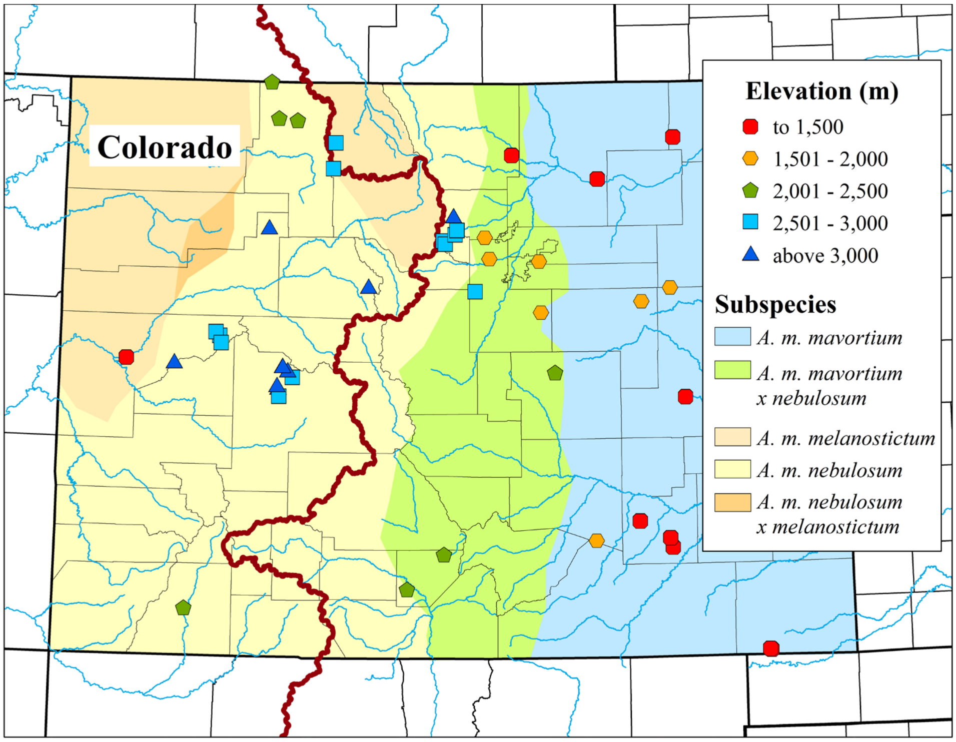

Figure 1. Distribution of subspecies of the Western Tiger Salamander (*Ambystoma mavortium*) in Colorado, including areas of intergradation, based on Figure 1 in Reese (1969). Blue background: *A. m. mavortium*; Green background: *A. m. mavortium* × *nebulosum*; Yellow background: *A. m. nebulosum*; Light tan background: *A. m. melanostictum*; Medium tan background: *A. m. nebulosum* × *melanostictum*. Symbols are sites where *A. mavortium* eggs were observed or described; they are coded by elevation (e.g., red octagons = lowest, blue triangles = highest). The red line depicts the Continental Divide and blue lines represent significant rivers and creeks.

developmental stages, comparisons of ovum diameters were not feasible; consequently, I do not include information about ovum diameter. In cases where I encountered eggs while the developing gamete was still roughly spherical, I sometimes was able to judge egg color. I used Pearson's Product Moment Correlation ($\alpha = 0.05$) to determine correlations between elevation and Julian date and between elevation and egg diameter.

Results

The 134 eggs that I measured ranged in diameter from 3.9–16.7 mm, with the largest egg diameters observed at high elevations (Table 1). I used records of *Ambystoma mavortium* eggs from 46 unique sites (27 personal records, 20 records from other sources, with one site having information from both personal observations and literature sources), throughout Colorado at elevations ranging between 1,322–3,281 m. Reports of eggs were found at breeding site elevations up to 3,410 m (Sexton and Bizer 1978). The localities represent each of the three subspecies present (*A. m. mavortium*, *A. m. nebulosum*, and *A. m. melanostictum*) in Colorado as well as the zone of intergradation between *A. m. mavortium* and *A. m. nebulosum*.

There were distinctly different patterns of egg presence related to elevation (Fig. 2). At elevations below about 2,000 m (the approximate elevation at which the foothills begin along the Front Range), eggs have been observed from 1 March to 21 August. There was no significant correlation between elevation and Julian date for sites at elevations below 2,000 m ($r = -0.276$, $t = -1.200$, df = 19, $P > 0.050$). At sites above 2,000 m, the earliest reports

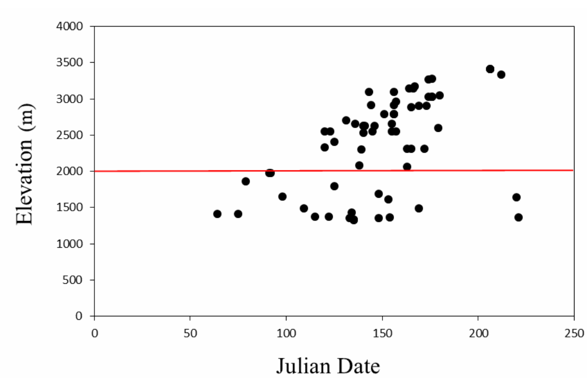

Figure 2. Relationship between elevation and Julian date at which the presence of eggs of Western Tiger Salamanders (*Ambystoma mavortium*) was noted at 67 breeding sites (pers. obs.; Steve Warren, pers. comm.) and literature sources (Hamilton 1949; Reese 1969; Sexton and Bizer 1978; Kiesecker and Clarke 1991; Lambert 2001). The red line at 2,000 m represents the approximate boundary between low elevation and montane sites. Where eggs were observed on different dates, the same site may be represented multiple times.

Table 1. Egg diameters of salamanders in Colorado by county. Subspecies codes are m = *A. m. mavortium*, m × n = *A. m. mavortium × nebulosum,* and n = *A. m. nebulosum*. Sites represented by images in Figure 3 are indicated in the Image column. The abbreviation SE = standard error.

County	Elevation (m)	Subspecies	Range (mm)	Mean and SE (mm)	n	Image
Otero	1,355	m	5.0–9.0	7.6 ± 0.40	10	
Otero	1,361	m	5.0–6.9	6.2 ± 0.42	4	3B
Otero	1,432	m	5.0–9.0	7.2 ± 0.51	10	
Lincoln	1,490	m	5.0–5.4	5.2 ± 0.09	4	3E
Lincoln	1,617	m	3.9–5.4	4.5 ± 1.77	8	
Boulder	1,793	m × n	8.4–8.7	8.5 ± 0.06	4	3G
Jefferson	1,975	m × n	4.2–5.4	4.5 ± 0.18	6	3H
Alamosa	2,334	m × n	6.8–7.1	6.9 ± 0.15	2	3J
Boulder	2,553	m × n	10.3–12.8	11.0 ± 0.38	6	
Mesa	2,632	n	6.4–10.4	8.4 ± 0.19	30	
Mesa	2,632	n	6.3–11.8	9.0 ± 0.23	38	
Gilpin	2,790	m × n		12.0	1	
Gilpin	2,960	m × n	9.3–16.9	12.4 ± 1.35	6	
Gilpin	2,960	m × n	13.1–16.2	14.6 ± 0.55	5	3O

of eggs were 30 April in both 1947 and 2006 (Hamilton 1949; pers. obs.) and the latest 31 July 1975 (Sexton and Bizer 1978). For breeding areas above 2,000 m, elevation and Julian date were significantly correlated ($r = 0.657$, $t = 4.339$, df = 44, $P < 0.010$).

I observed striking variations in the deposition mode and appearance of eggs across region and elevation (Fig. 3). Of 34 sites, five sites had eggs on both the substrate and submerged vegetation, seven sites had eggs on the substrate only, and 19 sites had eggs on submerged vegetation. For example, the only eggs I found at a breeding site were in a cluster of four that had been deposited in shallow water directly on the substrate (Fig. 3G). Detectability of eggs located on substrate likely was hindered with increasing water depth or turbidity. Most eggs are attached to a long stick raised slightly above the substrate, but several eggs are scattered directly on the substrate (Fig. 3D). When attached to branches or plant stems, eggs could be deposited singly or in short linear rows. Eggs sometimes are spaced along stems well above the substrate. One pond in western Boulder County had an especially distinctive example of egg deposition in which the eggs with large jelly layers were crowded along a stem several cm above the substrate (Fig. 3L).

Although I encountered eggs at different developmental stages, my impression was that ova from at least some low elevation sites seemed to be much lighter in color than ova encountered at high elevation sites, which were generally black. These light ova from low elevations were not dead and appeared to be developing normally. The amount of jelly surrounding the ovum or embryo was distinctly variable, with high elevation eggs having a much larger diameter (ovum or embryo and jelly layers) than eggs from low elevations (Fig. 4). There was a significant correlation between elevation and egg diameter for eggs measured from 14 breeding sites ($r = 0.611$, $t = 14.805$, df = 132, $P < 0.010$).

Discussion

At elevations below 2,000 m in eastern Colorado, most populations are *A. m. mavortium* or intergrades with *A. m. nebulosum* (Reese 1969). The earliest instances of egg deposition occur when ponds already contain water and ambient temperatures are high enough to clear ice from the surface of the pond as well as allow overland movement to the pond by salamanders from their overwintering sites. In contrast, more temporary sites often require rainfall to fill pools with sufficient water for breeding and egg deposition. Two instances of late season egg deposition occurred in August at temporary, low elevation sites after the first sufficient rainfalls of the year (Reese 1969; pers. obs.). This pattern is consistent with the timing of reproduction observed in *A. m. mavortium* in New Mexico (Webb and Roueche 1971; Jones and Collins 1992). The occasional presence of larvae of different size classes in a pond suggests egg deposition, presumably by different females, can occur asyncronously during the activity season (Hammerson 1999; pers. obs.).

The mountains of Colorado are occupied by both *A. m. nebulosum* and *A. m. melanostictum*, the intergrade populations of *A. m. nebulosum × mavortium*, as well as the limited area of intergradation of *A. m. nebulosum × melanostictum* (Reese 1969). Breeding of *A. m. nebulosum* in the mountains of Arizona and New Mexico takes place in the spring but apparently not later in the season (Jones and Collins 1992). In the mountains of

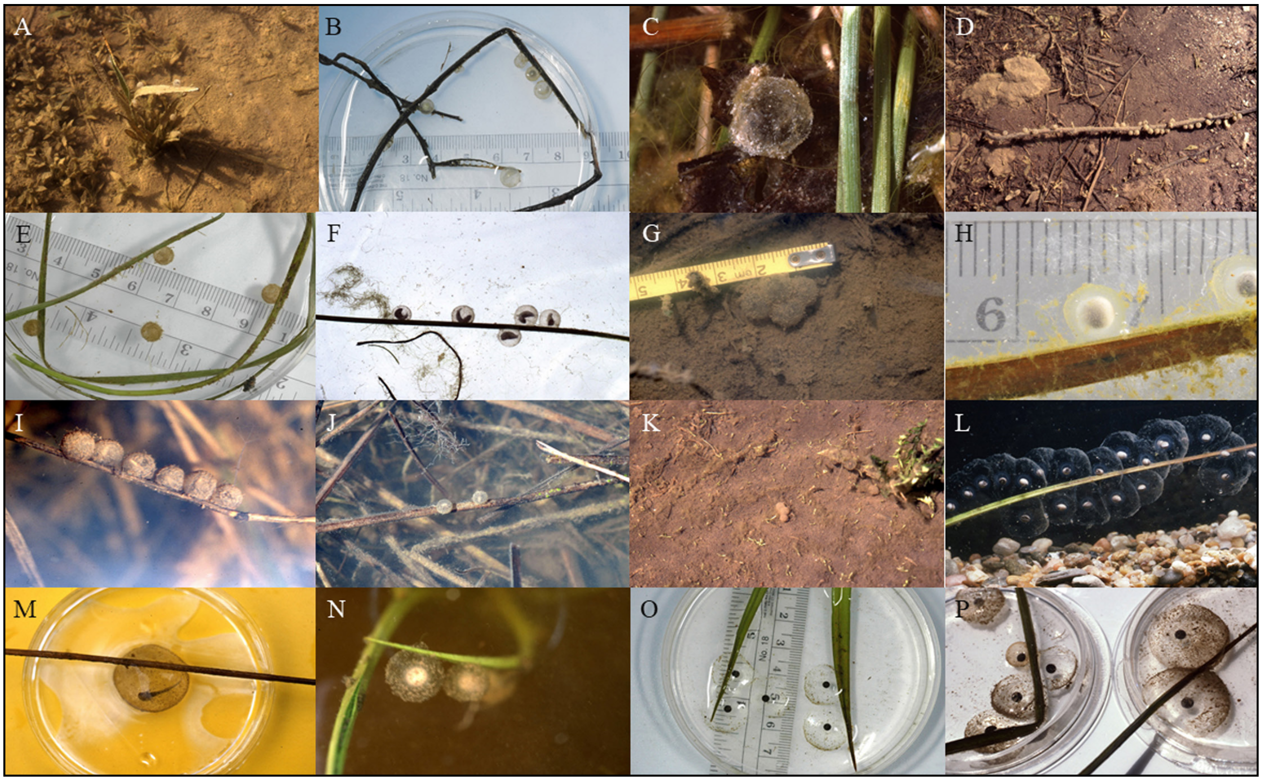

Figure 3. Representative eggs of Western Tiger Salamander (*Ambystoma mavortium*). (A) Otero County, 1,355 m elevation. (B) Otero County, 1,361 m elevation. (C) Weld County, 1,370 m elevation. (D) Otero County, 1,432 m elevation. (E) Lincoln County, 1,490 m elevation. (F) Lincoln County, 1,617 m elevation. (G) Boulder County, 1,793 m elevation. (H) Jefferson County, 1,975 m elevation. (I) La Plata County, 2,084 m elevation. (J) Alamosa County, 2,334 m elevation. (K) Mesa County, 2,528 m elevation. (L) Boulder County, 2,553 m elevation. (M) Jefferson County, 2,598 m elevation. (N) Boulder County, 2,656 m elevation. (O) Jackson County, 2,701 m elevation. (P) Gilpin County, 2,960 m elevation. Fig. 3A, 3C, 3D, 3G, 3I, 3J, 3K, and 3N photographed *in situ*. (Photographed by Lauren J. Livo).

Colorado, *A. mavortium* breeding sites generally begin the season with sufficient water as a result of snowmelt even if the sites dry up later, but elevation and other factors, including the source of water, water volume, and amount of solar radiation on the water body (Sexton and Bizer 1978), affect the thermal regime. Elevation and date are strongly correlated for montane sites, with earlier deposition at sites closer to 2,000 m elevation and with egg deposition later in the season at higher elevation sites.

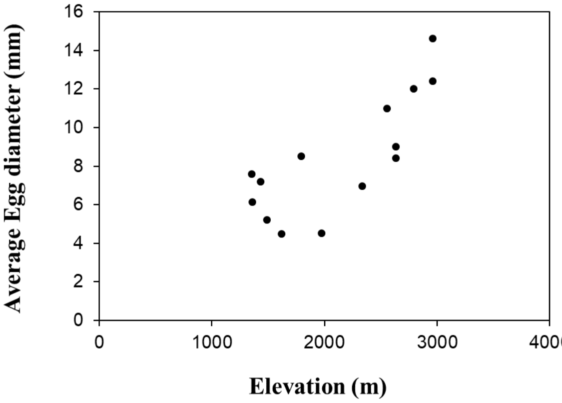

Figure 4. Relationship between average egg diameter (ovum or embryo and jelly layers) of Western Tiger Salamanders (*Ambystoma mavortium*) and elevation for 14 localities.

The most comprehensive review of *A. mavortium* in Colorado, including information about eggs, was provided by Reese (1969). He described eggs as having three jelly layers, generally being deposited singly or in a row, and having an average total diameter (ovum and surrounding jelly layers) of 5.5 mm, but he did not describe variation across the state (Reese 1969). Altig and McDiarmid (2015) state that *A. mavortium* has egg diameters ranging from 5.0–12.0 mm. Egg diameters observed by Hamilton (1949) from the mountains of Boulder County varied from 5 to 18 mm.

The eggs I measured varied substantially, with the lowest egg diameters being smaller than previously reported and the largest approaching the largest reported. Although Reese (1969) considered the diameter of 18 mm stated by Hamilton (1949) to be a *lapsus*, I measured egg diameters that approached 18 mm so I consider the measurement by Hamilton to be valid. Eggs from areas inhabited by *A. m. mavortium* had the smallest diameters, and *A. m. mavortium* × *nebulosum* and *A. m. nebulosum* the largest diameters.

Eggs sometimes are deposited on the bottom of the pond, but when available, most eggs are observed on sticks, plant stems, or other structures. Shallow, clear water likely increased the opportunity to detect eggs on substrate, while deep, stained, or turbid water reduced detection of eggs on the substrate. This agrees with observations by others (Hamilton 1949; Reese 1969; Wissinger and Whiteman 1992) including egg deposition in populations in Utah (Tanner et al. 1971), New Mexico (Collins et al. 1980), and southeastern Idaho (Jones et al. 2005). With respect to the mode of egg deposition, the eggs deposited by *Ambystoma mavortium* in the mountains of Boulder County are of particular interest. Hamilton (1949) collected eggs from two ponds in this area as part of his study of polydactyly at one of the ponds. He provided descriptions and photographs of a more typical linear arrangement of eggs along stems as well as what he described as a cluster of eggs, likely from Mud Lake, which he described as enclosed in a large mass of gelatinous material. Nearly half a century later, I observed eggs at Mud Lake with large jelly layers crowded along stems in a manner that could be described as an egg cluster. This is the only site at which I have observed eggs deposited in this manner. I observed the more typical deposition mode of eggs singly or in linear rows along stems at three breeding sites in the mountains within 3.8–14 km of Mud Lake.

In *A. mavortium* eggs that I observed, the amount of jelly surrounding the ovum or embryo increased with increasing elevation of the breeding site. This increase in egg jelly with elevation may be the result of several potential selective factors because amphibian egg jelly is thought to be protective in several possible ways, including: permitting or blocking fertilization (Elinson 1974); protecting or delaying desiccation when water levels drop (Marco and Blaustein 1998); protecting against water molds (Gomez-Mestre et al. 2006); protecting against ultraviolet radiation (Calfee et al. 2006); and making predation by both vertebrates and invertebrates more difficult (Werschkul and Christensen 1977; Ward and Sexton 1981; Pearl 2003). Although at least some light colored ova were observed at low elevation breeding sites, at most high elevation sites the ova I observed were heavily pigmented. The melanin in dark ova can be protective against ultraviolet radiation (Licht 2003).

Deposition of eggs on stems, leaves, and other structures may also be protective, especially against predation. Because I often found relatively few eggs compared to the expected clutch size of up to 666 eggs (Moore and Whiteman 2016), eggs may have been deposited in a variety of ways that I did not observe. Although I occasionally observed egg deposition on the substrate, especially in temporary pools at low elevations that at least initially would lack most egg predators, I did not observe egg deposition on the substrate at high elevation sites. High elevation breeding sites often had permanent water, potentially resulting in a greater existing load of egg predators such as leeches, beetle larvae, and even paedomorphic *A. mavortium*, which at least in the laboratory are known to cannibalize conspecific eggs (Hamilton 1948).

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Lauren J. Livo for several years conducted research on the Boreal Toad (*Anaxyrus boreas*) in collaboration with the Colorado Division of Wildlife. After receiving a Ph.D. from the University of Colorado, Boulder, USA, she continued her work on the Boreal Toad as a Post-Doctoral Fellow at the University of Colorado. Subsequent to retiring, she has been documenting the geographic distribution and phenology of amphibian and reptile species in Colorado, especially that of various introduced species including Pond Slider (*Trachemys scripta*), Colorado Checkered Whiptail (*Aspidoscelis neotesselatus*), and Chihuahuan Spotted Whiptail (*A. exsanguis*). (Photograph by Steve Wilcox).