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Reproductive biology of *Ambystoma* salamanders in the southeastern United States

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Abstract. Reproductive aspects of *Ambystoma* salamanders were investigated at sites in Louisiana (2010–12) and Mississippi (2013). Three species occurred at the Louisiana site, Spotted Salamander (*A. maculatum*), Marbled Salamander (*A. opacum*), and Mole Salamander (*A. talpoideum*), whereas only Spotted Salamanders were studied at the Mississippi site. A total of 162 and 71 egg masses of Spotted Salamanders were examined at the Louisiana and Mississippi sites, respectively. Significantly more Spotted Salamander eggs per egg mass were observed at the Mississippi site ($\bar{x}$ = 78.2) than the Louisiana site ($\bar{x}$ = 53.8; $P < 0.001$). The mean snout–vent length of female Spotted Salamanders at the Mississippi site (82.9 mm) was significantly larger than the Louisiana site (76.1 mm; $P < 0.001$). Opaque Spotted Salamander egg masses were not found at the Mississippi site, but accounted for 11% of examined egg masses at the Louisiana site. The mean number of eggs per egg mass at the Louisiana site did not differ between opaque (47.3) and clear (54.6) egg masses ($P = 0.21$). A total of 47 egg masses of the Mole Salamander were examined, with a mean number of 6.7 embryos per mass. Twenty-three individual nests of the Marbled Salamander were found either under or in decaying logs in the dry pond basins. There was no difference between the mean numbers of eggs per mass of attended nests (93.0) versus those that were discovered unattended (86.6; $P = 0.67$). Females tended to place their nests at intermediate heights within the pond basin.

Keywords. *Ambystoma maculatum*, *A. opacum*, *A. talpoideum*, reproduction, clutch size, nest site selection, Louisiana, Mississippi

Introduction

Ambystoma is a genus of North American salamanders in the family Ambystomatidae, known collectively as the mole salamanders. Though there are some neotenic examples, most *Ambystoma* are terrestrial, spending the majority of their time in underground burrows. They are nocturnal and most typically observed during their reproductive season when they emerge from underground at night and migrate to their breeding ponds. Most terrestrial *Ambystoma*, such as the Spotted Salamander, *A. maculatum* (Shaw, 1802) and the Mole Salamander, *A. talpoideum* (Holbrook, 1838), lay their eggs in winter or early spring in ponds that have already filled with water (Fig. 1). However, a few species, including the Marbled Salamander, *A. opacum* (Gravenhorst, 1807), lay eggs in the fall in a constructed nest in the dry pond basin (or at the periphery of a reduced pond) before it fills (Fig. 1; Dunn, 1917; Petranka and Petranka, 1981a).

A single female Spotted Salamander typically lays 2–4 masses of eggs per year. The large, firm globular egg masses, each typically containing 1–250 eggs, are laid in relatively shallow areas of the pond either attached to vegetation or resting on the pond bottom (Bishop, 1941; Shoop, 1974). Mole Salamanders lay eggs singly in Atlantic Coastal Plain populations, but in Gulf Coastal Plain populations a single female lays many small delicate clusters of eggs, usually attached to vegetation (Semlitsch and Walls, 1990; Trauth, 2005). Although it is very difficult to determine the female responsible for a particular egg mass in Spotted and Mole Salamanders,

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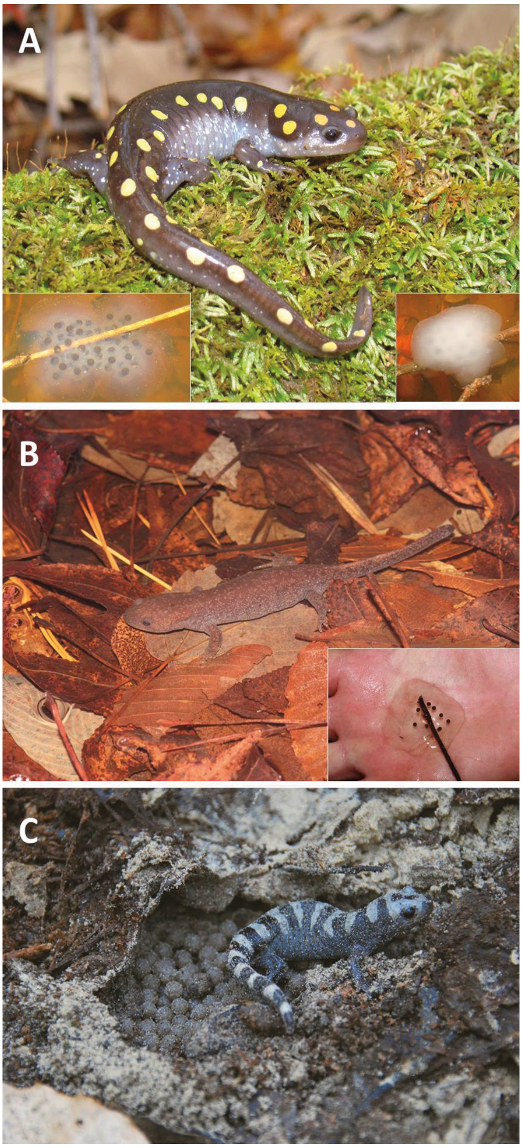

Figure 1. The three salamander species and their eggs that are discussed in this paper. (A) Spotted Salamander (*Ambystoma maculatum*), clear egg mass (inset lower left), and opaque egg mass (inset lower right). (B) Mole Salamander (*A. talpoideum*) in water, egg mass (inset lower right). (C) Marbled Salamander (*A. opacum*) guarding her clutch that was laid under a log. Photos in (A) and (C) by Brad M. Glorioso. Photos in (B) by Jeromi Hefner.

the entire clutch (30–200 eggs) of a female Marbled Salamander is laid at once, typically under a log or other cover, and attended by the female for some time thereafter (Petranka, 1998).

The Marbled Salamander is unique in that it is the only *Ambystoma* species that exhibits parental care in the form of nest-guarding (Nussbaum, 1985, 1987). Female nest site selection may be influenced by factors such as microsite elevation within the pond basin (Petranka and Petranka, 1981a; Jackson et al., 1989), pond hydrology (Wojnowski, 2000), cover availability (Petranka and Petranka, 1981b; Figiel and Semlitsch, 1995), and soil moisture (Figiel and Semlitsch, 1995; but see Marangio and Anderson, 1977). The length of time that females brood their clutch is variable, but females may leave the clutch before inundation (McAtee, 1933; Jackson et al., 1989; Petranka, 1990). Although the mechanism is not fully known, it appears nest brooding in Marbled Salamanders enhances embryonic survival, perhaps by defending against predators, fungal invasion, and desiccation (Petranka and Petranka, 1981b; Jackson et al., 1989; Petranka, 1998; Croshaw and Scott, 2005).

We report clutch and egg mass data and other aspects of reproduction of three species of *Ambystoma* salamanders. Our data span two and a half breeding seasons at a site in Kisatchie National Forest in Louisiana and one breeding season at a site along the Natchez Trace National Parkway in Mississippi. Though limited in scope, the data reported herein contributes to the reproductive knowledge of these species.

Materials and Methods

We conducted the work reported herein primarily in the Kisatchie District of Kisatchie National Forest in Natchitoches Parish, Louisiana, USA, but also along the Natchez Trace National Parkway in Hinds County, Mississippi, USA (Fig. 2). In Kisatchie, we began our examination of the ambystomatid salamander community in the fall of 2010 at two nearby ponds and associated wetland areas within the Kisatchie Bayou Recreation Area (Fig. 3). The study area in Kisatchie has not been logged in over 100 years and is forested with large hardwoods and pines. The study area is bordered on the west by Kisatchie Bayou (a 7^{th} order stream), on the east by steep hills, and to the north and northeast by a large clearcut (Fig. 3).

Mole Pond at the Kisatchie site is a large, relatively deep (~2 m at its deepest) ephemeral pond with ample leaf litter and coarse woody debris. Several large pine (*Pinus* sp.) and gum (*Nyssa* sp.) trees are at the edges and shallower portions of the pond, with buttonbush (*Cephalanthus occidentalis*) occupying the deepest portions. Ox Pond is a deeply cut old oxbow of Kisatchie Bayou, and is filled with bald cypress (*Taxodium*

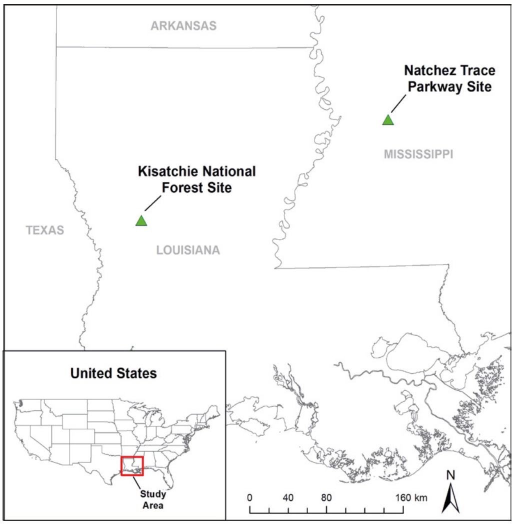

Figure 2. Location of the study areas within Louisiana and Mississippi, USA.

distichum) throughout the pond basin. Whereas Mole Pond typically fills with rainwater and runoff from the nearby hills, Ox Pond typically fills from flooding of the bayou. The third major breeding area at the Kisatchie site is Ditch, a relatively shallow (depth < 1 m) sinuous intermittently flooded waterway that is connected to Ox Pond and travels through the middle of the study area. Other depressions in the study area are insignificant to *Ambystoma* reproduction.

The pond at the Natchez Trace site is man-made, rectangular in shape, and surrounded by a low berm. Oak (*Quercus* sp.) dominates the surrounding forest. The water in the breeding pond is permanent, which is unusual with respect to typical breeding ponds used by *Ambystoma*, but is suitable for breeding because it lacks fish predators. The pond is relatively small compared to the Kisatchie ponds, with moderate depth (> 1 m at its deepest), and is covered with duckweed (*Lemna* sp.) on the surface, with a very soft bottom.

We searched for ambystomatid salamanders at the Kisatchie site primarily by turning logs both within the dry pond basins and the associated upland areas. We also used unbaited minnow traps to capture adult salamanders within the subsequently flooded ponds. We sexed and measured each salamander for snout–vent length (SVL), total length (TL), and body mass before injecting them with a Passive Integrated Transponder (PIT) tag. At the Natchez Trace site, we used a drift fence that encircled the breeding pond to capture Spotted Salamanders migrating into the pond in 2013. We sexed and measured each salamander for SVL, TL, and body mass before injecting them with Visible Implant Elastomer (VIE) batch marks as part of a larger study on salamander migration.

We discovered egg clutches of the Marbled Salamander in Kisatchie by turning logs in the bottom of Mole and Ox ponds as well as Ditch. When a clutch was found attended by a female, we processed the female as stated above and placed the entire egg clutch into a pour-boat. With the pour-boat angled downwards at the clutch site, we counted the eggs as we carefully returned them singly or in small groups back to the oviposition site. We then returned the female to her clutch, and carefully replaced the log. We noted the location with colored pin flags marked with the nest number we assigned. On

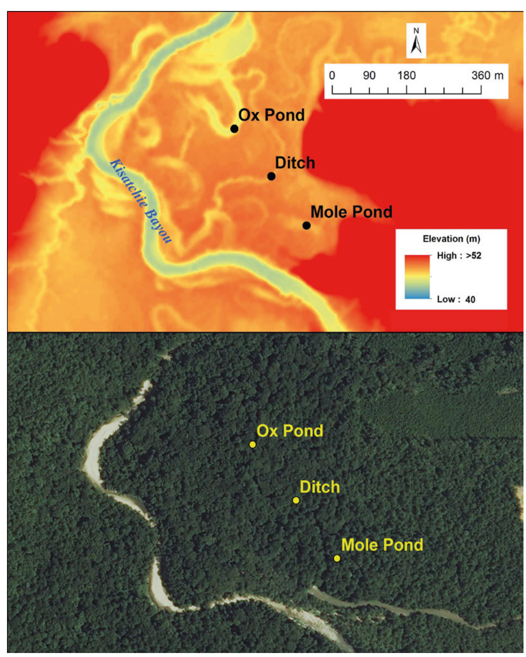

Figure 3. Elevation of the study site showing the locations of the primary *Ambystoma* breeding sites in Kisatchie National Forest and their relative position to Kisatchie Bayou to the west and the hills to the east (top) and aerial imagery of the study site showing the clearcut to the northeast (bottom). The scale applies to both images.

Table 1. Data on the numbers of eggs per mass (or clutch size) for *Ambystoma* species at Kisatchie National Forest (K) and Natchez Trace Parkway study sites (NT).

Species	# of egg masses	Mean # of eggs per mass	SD	Range
A. maculatum (K)	162	53.8	23.12	3–111
Clear	144	54.6	23.30	5–111
Opaque	18	47.3	21.15	3–81
A. maculatum (NT)	71	78.2	34.02	13–147
A. talpoideum (K)	47	6.7	3.43	2–15
A. opacum (K)	23	90.8	33.41	52–175
Attended	15	93.0	30.91	54–152
Unattended	8	86.6	39.59	52–175

subsequent trips we would check on the status of the nest by gently lifting the log to observe if the female and/or the eggs were still present.

We used a laser level to survey the pond basin bathymetry of Mole Pond during a time when it was completely dry. An automatic water depth logger was placed at the deepest point in the pond and the location of all marbled salamander nests were recorded by measuring the bearing and distance from the deepest point. With these data we were able to reference the locations of all Marbled Salamander clutches we found relative to the depth of the pond. The depth of the water at any nest at any time could then be determined with geographic information software by using the bathymetry data combined with the water depth.

Mole Salamander egg masses were not our primary interest, and nearly all masses examined were found on a single day (19 January 2012) while looking for Spotted Salamander egg masses, our primary target. Whereas we were able to easily count the number of embryos in the small egg masses of the Mole Salamander in the water, the larger, firmer egg masses of the Spotted Salamander generally required a different approach. At both the Kisatchie and Natchez Trace study sites we used a technique modeled after Harris (1980) to count the number of embryos in the egg masses of Spotted Salamanders (Glorioso et al., 2012). We gently pressed the egg mass between a top, clear plastic tray and a bottom tray that was marked with black lines 1 cm apart and then spray-painted white on the bottom. We then took digital photographs of each mass and counted the number of embryos using photo-editing software.

All statistical tests were performed in the R statistical software environment (R Core Team, 2012). Comparisons of means between two groups were conducted using *t*-tests, and ANOVA was used for comparisons involving a factor with three levels. Linear regression was used to model the correlation between size of female Marbled Salamanders and the number of eggs they attended. Significance for all tests was set at $\alpha = 0.05$.

Table 2. Data on snout-vent length (SVL) for *Ambystoma* species at Kisatchie National Forest (K) and Natchez Trace Parkway study sites (NT).

Species	# of unique females	Mean SVL (mm)	SD	Range
A. maculatum (K)	22	76.1	3.91	69–84
A. maculatum (NT)	90	82.9	5.60	64–96
A. talpoideum (K)	11	56.2	2.44	51–59
A. opacum (K)	15	56.7	3.99	52–64

Results

In Kisatchie, none of the Marbled Salamander nests in 2010–11 hatched as a severe drought prevented filling of the breeding ponds (NOAA, 2011). In 2011–12 the ponds filled, but there was a severe flood in late March 2012 that introduced fish and turtles into the ponds while also washing away most of the leaf litter substrate of the breeding ponds. Marbled Salamander larvae in 2011–12 were quite large by the time of the flood, and some may have transformed prior to the flood. Marbled Salamanders likely had better survival than Spotted and Mole Salamanders, which were either unhatched or very small larvae at the time of the flood. The ponds dried in the summer of 2012 and because we ceased the study in the fall of 2012, we were unable to investigate breeding in 2012–13 except in Marbled Salamanders that had already laid their nests.

We were able to track breeding in Marbled Salamanders for three seasons, whereas we could only track breeding in Spotted and Mole Salamanders in one (2011–12). There were some marked differences noticed between the breeding ponds. Whereas it seemed that both Mole and Ox Pond had large numbers of Marbled and Mole Salamanders based on eggs, Spotted Salamanders did not show the same result. Spotted Salamanders used Mole Pond to a much greater extent, with over 70% of the total egg masses of that species found there. We discovered only three egg masses in Ox Pond. Ditch

Table 3. Reproductive data on all Marbled Salamander nests in Mole Pond (M), Ox Pond (X), and Ditch (D) observed from Fall 2010–Fall 2012 at Kisatchie National Forest. The fate of nests is indicated by a plus sign (+) when nests presumably hatched and by a minus sign (–) when hatching did not occur, due to drought.

Nest ID	Date First Observed	Attended	Date last observed attending	Minimum # of days attending	# of eggs	Nest Fate	Pseudo Elevation (cm)	Comments
M01	10 Nov 2010	Yes	7 Dec 2010	27	85	–	109.88	
M02	10 Nov 2010	Yes	21 Dec 2010	41	152	–	105.77	
M03	10 Nov 2010	Yes	21 Dec 2010	41	126	–	88.09	
M04	10 Nov 2010	Yes	16 Nov 2010	6	100	–	85.95	
M05	21 Dec 2010	Yes	21 Dec 2010	1	72	–	97.54	
M06	21 Dec 2010	Yes	21 Dec 2010	1	227	–	36.88	2 nests, two attending females; one same as M14
M07	21 Dec 2010	Yes	21 Dec 2010	1	237	–	66.45	2 nests, one attending female
M08	21 Dec 2010	Yes	21 Dec 2010	1	79	–	57.61	
M09	5 Jan 2011	No	-	-	57	–	146.30	
M10	5 Jan 2011	No	-	-	82	–	146.30	
M11	5 Jan 2011	No	-	-	57	–	150.57	
M12	14 Dec 2011	Yes	14 Dec 2011	1	54	+	33.67	
M13	18 Oct 2012	Yes	8 Nov 2012	20	122	+	51.18	
M14	25 Oct 2012	Yes	8 Nov 2012	13	59	+	75.91	Same attending female as M06
M15	8 Nov 2012	Yes	8 Nov 2012	1	77	+	67.17	
M16	8 Nov 2012	Yes	12 Dec 2012	34	69	+	99.15	
X01	16 Nov 2010	Yes	21 Dec 2010	35	125	–	-	
X06	16 Nov 2010	No	-	-	52	–	-	
X15	21 Dec 2010	Yes	21 Dec 2010	1	79	–	-	
X08	25 Jan 2011	No	-	-	175	–	-	
X09	10 Feb 2011	No	-	-	85	–	-	
X16	14 Dec 2011	Yes	14 Dec 2011	1	60	+	-	
X17	14 Dec 2011	Yes	14 Dec 2011	1	129	+	-	
X18	18 Oct 2012	Yes	18 Oct 2012	1	106	+	-	
D01	25 Oct 2012	Yes	25 Oct 2012	1	86	+	-	

was used by many Spotted (over 26% of the total egg masses found) and Mole Salamanders for reproduction, but was not used much by Marbled Salamanders. Only one nest of a Marbled Salamander was found in Ditch, but there was very little coarse woody debris available in Ditch and nests may have escaped detection under leaf litter.

A total of 162 egg masses of Spotted Salamanders were counted in Kisatchie in early 2012. At Natchez Trace, 71 Spotted Salamander egg masses were counted in early 2013. The number of eggs within Spotted Salamander egg masses was significantly larger at the Natchez Trace population compared to the Kisatchie population (t = –5.51, df = 99, P < 0.001; Table 1). Likewise, the mean SVL of adult female Spotted Salamanders at the Natchez Trace population was significantly larger than adult females in the Kisatchie population (t = –6.64, df = 45, P < 0.001; Table 2). Whereas no Spotted Salamander egg masses were opaque in the Natchez Trace population, opaque masses accounted for 11.1% of total egg masses in the Kisatchie population (Table 1). The mean number of eggs in opaque masses was slightly smaller than in clear masses, but this difference was not significant (t = –1.36, df = 22.5, P = 0.189; Table 1).

Hundreds of Mole Salamander egg masses were observed in Mole and Ox Pond, as well as Ditch in early 2012. However, as it was not a priority of ours, we only examined 47 egg masses, mostly from Mole Pond. The mean number of eggs per mass was 6.7 (Table 1). Only 11 adult female Mole Salamanders were measured and they had a mean SVL of 56.2 mm (Table 2).

In Kisatchie, 16, three, and six Marbled Salamander clutches were found under or in decaying logs in the 2010–11, 2011–12, and 2012–13 breeding seasons,

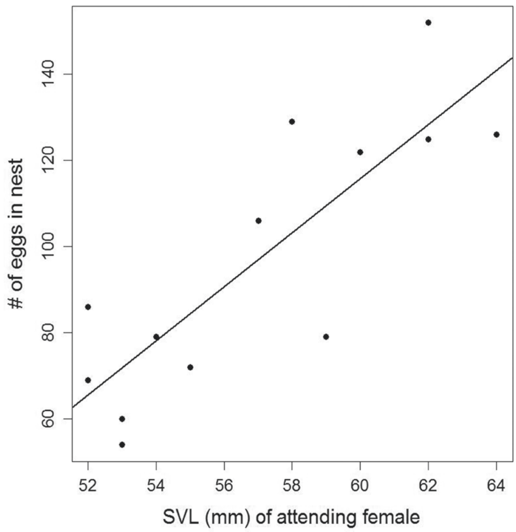

Figure 4. The relationship between the SVL (mm) of nest-attending Marbled Salamanders and the number of eggs in their clutch (slope = 6.29, $r^2 = 0.67$).

respectively (Table 3). Two of the 16 clutches found in 2010–11 were believed to be communal clutches from two females, as they were laid in such close proximity to each other it was difficult to delineate them; the eggs were counted together for both of those cases. Of the 23 clutches attributable to a single individual, the mean number of eggs per clutch was 90.8 (Table 1). There was no significant difference in clutch size found between 15 nests first discovered attended by a female ($\bar{x} = 93$) from eight nests first discovered unattended ($\bar{x} = 86.6$, $t = 0.40$, $df = 11.7$, $P = 0.70$). Also, there was no significant difference in mean clutch size by season ($F = 0.26$, $P = 0.78$). The mean SVL of females found attending nests was 56.7 mm (Table 2), with SVL and clutch size exhibiting a significant positive correlation ($r^2 = 0.67$, $P < 0.001$; Fig. 4).

In Mole Pond in Kisatchie, Marbled Salamander nests from all years combined had a mean pseudo elevation of 88.7 cm above the deepest part of the pond (SD 37.07, range 33.7–150.6; Fig. 5). The highest part of the pond that is typically flooded at full pool is about 206 cm; therefore, Marbled Salamanders tended to deposit their eggs at intermediate heights within the pond basin (Fig. 5). One female was found attending a nest in multiple years; she was part of one of the communal nests in 2010–11 and was found attending a nest in 2012–13 about 26 m away and twice the pseudo elevation of her prior nest in 2010–11.

Nineteen of the 27 total Marbled Salamander nests were initially attended by a female when discovered. Eleven of those 19 attended nests were abandoned by the female by the subsequent visit to the site, which usually was a week later, but sometimes longer. Of the

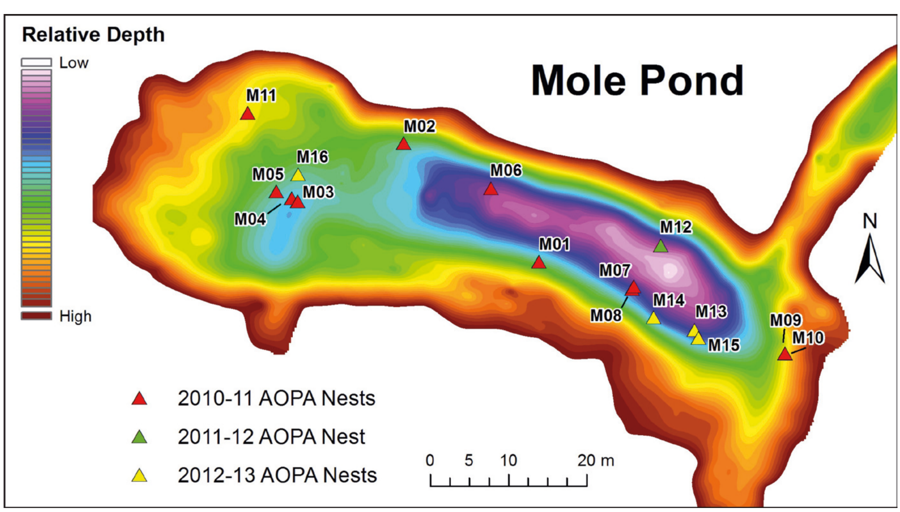

Figure 5. Relative depth of egg clutches laid by Marbled Salamanders in Mole Pond in the three breeding seasons. Approximately 2 m separates the lowest point of the pond from the highest point at full pool.

eight nests where females remained at least until the next visit, six were still attending the nest 20 or more days later ($\bar{x}$ = 27 days, SD 13.04, range 6–41; Table 3). The longest periods of brooding occurred in 2010–11 during a drought which prevented the ponds from ever filling. Only two of eight females in 2011–12 and 2012–13 were still attending on a subsequent visit, with all females abandoning their nests well before pond filling.

Discussion

Based on our experience with the pond prior to the study we knew that Mole Pond in Kisatchie was an important breeding area for all three *Ambystoma* species. Ox Pond, Ditch, and other nearby depressions were investigated after the study commenced upon an examination of elevation data for the area. We were unsure how important Ox Pond would be as it had wholly different characteristics than Mole Pond. Whereas both ponds exhibited good numbers of Marbled Salamander clutches and Mole Salamander egg masses, it was unexpected that Spotted Salamanders would almost totally disregard Ox Pond as a potential breeding pond, especially since we routinely found adults at the rim of the pond. It seems that many of the Spotted Salamanders in this area likely breed in Ditch. We are unable to hypothesize a good explanation as to why Spotted Salamanders rarely bred in Ox Pond (only three masses), especially since we did not detect noticeable differences in egg nests and masses in the other two species between Mole and Ox Ponds.

The mean number of eggs in Spotted Salamander egg masses from both our sites is on the low end of other reported studies, with the Kisatchie population being one of the lowest reported. Most reported mean number of eggs per mass have come from studies in the northern part of their range (e.g., Bishop, 1941; Talentino and Landre, 1991; Pinder and Friet, 1994; but see Trauth et al., 1995). It is conceivable that reduced female body condition due to the prolonged drought the year prior had a role in the low mean number of eggs per mass in the Kisatchie population.

Perhaps the differences in the mean number of eggs per mass observed can be attributed to differences observed in female size. In this study, we observed larger female size and greater mean number of eggs per mass in the Natchez Trace population compared to the Kisatchie population. It has been shown that Spotted Salamander clutch size (i.e., full complement of eggs produced in a season) is positively correlated with female SVL (Kaplan and Salthe, 1979; Wilbur, 1977; but see Shoop, 1974), though Woodward (1982) stated only one of his two sites exhibited this pattern. Unless directly observed or through genetic testing, the female responsible for a given egg mass goes unknown (Karraker and Gibbs, 2011). During this study, five gravid females from the Kisatchie population expelled their PIT tag within one of their egg masses. From this limited sample, a positive relationship of SVL to the number of embryos in an egg mass was observed (Glorioso et al., 2012). These aforementioned studies and findings in this study lend support to the idea that the differences observed between the two study sites can be attributed, at least in part, to differing female body size between populations.

Contrary to our results, Brodman (1995) and Metts (2001) both found significantly more embryos in opaque Spotted Salamander egg masses compared to clear egg masses. Hardy and Lucas (1991) stated that the embryos of opaque masses are not visible; however, we found it was possible to count the embryos of our opaque masses with the technique we used, though there was undoubtedly more uncertainty compared to counting embryos of clear masses. Though it is known that opaque masses are caused by hydrophobic proteins in the outer jelly layer, the functional role or advantage of this trait is unclear (Hardy and Lucas, 1991; Ruth et al., 1993). Spotted Salamander populations exhibit varying proportions of opaque masses whereas, in other populations, opaque masses seemingly do not occur at all.

Raymond and Hardy (1990) found egg masses of Mole Salamanders ranging from 3–50 eggs in a northwestern Louisiana population, with a mean of 17.4. Trauth et al. (1995) found 14–99 eggs per egg mass of Mole Salamanders with a mean of 41.1 eggs in an Arkansas population. Though these two studies had means higher than our largest examined egg mass, Bishop (1943) reported a range of 4–20 eggs per egg mass from a southeastern Louisiana population, which is similar to our findings. The number of eggs per egg mass in Gulf Coastal Plain populations is quite variable and dependent on the population (Trauth, 2005).

The mean number of eggs per clutch of Marbled Salamanders in this study (90.8) is similar to that of other studies in the southern part of their range (e.g., Petranka and Petranka, 1981b; Trauth et al., 1989; Scott, 1990). Clutch size in our study was positively correlated with SVL of the female, as has been found in several other studies (e.g., Walls and Altig, 1986; Petranka, 1990; Scott and Fore, 1995). However, some studies have not reported such a correlation between clutch size and SVL (Kaplan and Salthe, 1979; Trauth et al., 1989). One caveat to remember is that most clutch sizes are estimates of counts from eggs in the nest, and thus, some uncertainty exists that no eggs have been lost before finding the nest.

Unlike Petranka (1990) and Petranka and Petranka (1981b), we found that the clutch size of attended and unattended nests were not significantly different. This may be variable among populations and year. Alternatively, it could be explained by simple predation upon eggs of abandoned nests before discovery in prior studies.

The brooding times observed in our study are within the range observed in other studies. The longest times observed came from the drought year when the ponds did not fill. In some extreme northern populations where eggs are subject to freeze, brooding periods are short (Bishop, 1941). However, some females, especially in populations with a mild climate, may brood their nests for up to three months or more (e.g., Worthington, 1968; Hassinger et al., 1970; Petranka and Petranka, 1981a). Noble and Brady (1933) found viable eggs as late as March.

It is likely that the disturbances caused by the initial processing of both the female and her clutch, and subsequent ‘checks’ of the status of the clutch led to some of the abandoned nests we observed (Noble and Brady, 1933). In addition, wild hogs were observed in the area and are apparently abundant as evidenced by their activity in the pond basins. On several occasions, logs that had females brooding their clutches underneath were disturbed by apparent hog rooting. However, nest desertion prior to pond filling is common even in undisturbed nests (Noble and Brady, 1933). Females may leave their clutch within a few days or weeks after laying, and only occasionally remain with their clutches through hatching (Noble and Brady, 1933; Petranka, 1990). Unlike some plethodontid salamanders that exhibit well-developed brooding behavior, female Marbled Salamanders show much weaker affinities to their nests (Noble and Brady, 1933; Petranka, 1990). Females have been observed brooding inviable nests despite ample opportunities to disperse, and deserting viable nests, which suggest that females may not be fully able to discern nest viability (Petranka, 1990). There appears to be no increase in energetic cost to brooding, as foraging in both males and females ceases during breeding (Kaplan and Crump, 1978).

Nest site selection in Marbled Salamanders may affect larval growth, size-dependent interactions, and survival, and has been the topic of several investigations (Graham, 1971; Petranka and Petranka, 1981a; Jackson et al., 1989; Petranka, 1990). Our study found that nests were generally placed at intermediate depths within the basin of Mole Pond, with no nests being observed in the lower 30 cm or the upper 50 cm of the ~200 cm pond basin. However, because we did not attempt to locate nests under leaf litter, we cannot be sure that our results accurately represent nest site selection.

Intermediate nest placement, as found in this study, is similar to the findings of Petranka and Petranka (1981a) and Graham (1971). The rationale for intermediate nest placement is that if eggs are laid at the very bottom of a pond, they may hatch into shallow water, which may subsequently dry, killing all the larvae. Alternatively, nests placed at the top of pond basins run the risk of freezing in harsh climates or never being inundated in drier than normal years. Jackson et al. (1989) and Croshaw and Scott (2006), however, found nests concentrated at the lowest elevations of breeding ponds. Some of the variation observed could be due simply to cover availability, though Croshaw and Scott (2006) experimentally controlled for cover availability in the field in their study. In our study, however, there were sufficient logs at both the lowest and highest parts of the pond basin where we failed to ever detect a nest. Other variables correlated with elevation, such as temperature, soil moisture, soil organic matter, fine root biomass, and soil texture may be important cues used in nest site selection, and could explain some of the variation observed in nest site placement across studies (Croshaw and Scott, 2006).

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