

# A test of reproductive advantage in two competitive mole salamanders (Caudata: Ambystomatidae) from a single site in South-Central Pennsylvania

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Eleven female Jefferson Salamanders (*Ambystoma jeffersonianum*) and five female Spotted Salamanders (*A. maculatum*) were collected during 28 February-17 March 2011 from the same vernal pool in south-central Pennsylvania for comparison of reproductive characteristics. Mean clutch size (169.5 eggs) and relative clutch mass (12.9%) of *A. jeffersonianum* was larger than those values of *A. maculatum* (130.8 eggs and 9.4%, respectively) despite an 8.9% smaller body size of the former. However, mean egg diameter was larger in *A. maculatum*. Greater fecundity, even if at the cost of egg size, could provide *A. jeffersonianum* with an added reproductive advantage in areas of syntopy with *A. maculatum*, where it is known to be a superior competitor and predator of *A. maculatum* at the larval stage.

**Key words:** ambystomatids; egg size; females; Pennsylvania; reproduction.

**Un test de ventaja reproductiva en dos salamandras topo (Caudata: Ambystomatidae) capturadas en una única localidad del centro-sur de Pennsylvania.** Once hembras de la salamandra de Jefferson (*Ambystoma jeffersonianum*) y cinco de la salamandra moteada (*A. maculatum*) fueron capturadas entre el 28 de febrero y el 7 de marzo de 2011, en una charca vernal del centro-sur de Pennsylvania con el fin de comparar algunas características reproductivas. El tamaño medio de puesta de *A. jeffersonianum* fue de 169,5 huevos y la masa relativa de la puesta del 12,9%. Estos valores fueron mayores que los de *A. maculatum* (130,8 huevos y 9,4% respectivamente), a pesar de que *A. jeffersonianum* fuese un 8,9 % más pequeña que *A. maculatum*. Sin embargo, el diámetro medio de los huevos fue mayor en *A. maculatum*. La mayor tasa de fecundidad, aunque a expensas de un menor tamaño de los huevos, de *A. jeffersonianum* podría suponer una ventaja reproductiva añadida en áreas sintópicas con *A. maculatum*, donde se sabe que las larvas de *A. jeffersonianum* presentan una ventaja competitiva y depredan sobre las larvas de *A. maculatum*.

The family Ambystomatidae, commonly known as mole salamanders, is a not so diverse but conspicuously widespread and endemic North American clade (Frost *et*

*al.*, 2006). The Jefferson Salamander (*Ambystoma jeffersonianum*) and the Spotted Salamander (*A. maculatum*) are two of the most characteristic species in the Eastern



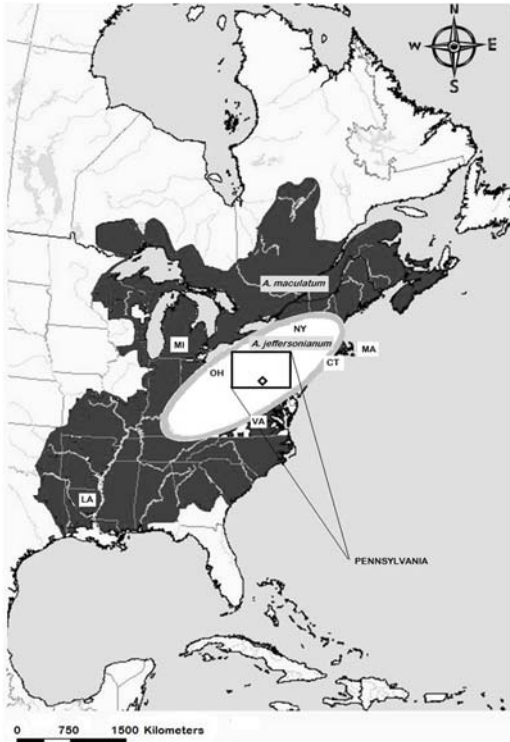
**Figure 1:** Typical (A) Jefferson Salamander (*Ambystoma jeffersonianum*) and (B) Spotted Salamander (*A. maculatum*) from south central Pennsylvania. Pictures by Pablo R. Delis.

North America region (CONANT & COLLINS, 1998) (Fig. 1). These two species briefly visit vernal pools usually in late winter and early spring to reproduce, with the Jefferson Salamander being the earlier breeder of the two species. The adults move back to upland habitats shortly after mating, and the larvae grow and metamorphose in syntopy in these aquatic habitats over the spring and early summer (HULSE *et al.*, 2001).

The geographic range of the Jefferson Salamander is subsumed by that of the

Spotted Salamander in Pennsylvania (HULSE *et al.*, 2001), and their populations reach approximately the middle of their respective geographic ranges (CONANT & COLLINS, 1998) (Fig. 2). The Jefferson Salamander is considered a state threatened species or at least species of special concern in part of its range (HAMMERSON, 2004; MULLIN & KLUEH, 2009). Some population descriptions such as specific breeding sites, adult sizes (HULSE *et al.*, 2001) and physical factors influencing egg deposition patterns of these two species have been explored for Pennsylvania (ROWE & DUNSON, 1993; HORNE & DUNSON, 1994). Unfortunately, still little is known about many aspects of the life history of these two species in Pennsylvania (HULSE *et al.*, 2001)

Reproductive output, in the form of newly metamorphoslings, and female fecundity, in the form of numbers of eggs produced, are two common ways of measuring reproductive success. Timing of newly metamorphoslings to emerge is the result of combined abiotic (temperature, pH, hydroperiod, etc.) (VIPARINA & JUST, 1975; JØRGENSEN, 1992; DUELLMAN & TRUEB, 1994) and biotic (food availability, predation, competition, etc.) (DUSHANE & HUTCHINSON, 1944; SALTHE & MECHAM, 1974) factors over the lives of the larvae. In some cases, interspecific interactions are critical to determine metamorphosing success. Studies on the larval interactions of the Jefferson and the Spotted Salamanders at their breeding sites revealed that the Spotted Salamander was eaten by the Jefferson Salamander (NYMAN, 1991), and that the former species responded to the presence of this predator by moving to warmer strata away from the Jefferson



**Figure 2:** Range distributions of the Jefferson Salamander (*Ambystoma jeffersonianum*) and the Spotted Salamander (*A. maculatum*) in North America. Highlighted states with pertinent biological data: Connecticut (CT), Louisiana (LA), Massachusetts (MA), New York (NY), Ohio (OH), and Virginia (VA). Diamond represents geographic location in this study.

Salamander larvae (STAUFFER *et al.*, 1983). Crowded conditions negatively impacted both species when alone and similarly so when together (BRODMAN, 1996). Fecundity, a strong measure of reproductive effort, is conventionally measured by the size of the egg and the numbers of eggs in each egg mass, the later commonly known as clutch size. Among salamanders, clutch size is generally associated with female body size, and the relationship between ovum size and female body size is not

consistent (KAPLAN & SALTHER, 1979). Egg counts for these species are typically known from egg masses at the breeding sites (PETRANKA, 1998; HULSE *et al.*, 2001; BRODMAN, 2005; SAVAGE & ZAMUDIO, 2005). Direct counts of ovarian eggs, however, do not exist for Pennsylvanian populations and are uncommon elsewhere in their range.

In light of the competitive superiority of larval Jefferson Salamanders over those of Spotted Salamanders, we explored the reproductive advantages of the breeding adults. Specifically, we measured this potential adult advantage by examining body size, female fecundity, and a relative measure of female reproductive effort, in this case relative clutch mass (RCM), at a breeding site in south-central Pennsylvania. We used an array of interconnected temporary pools, used historically by these species, during their 2011 breeding season of late winter-early spring. This study specifically compares clutch characteristics directly from ovarian egg counts and adult body sizes between these two species. These variables have never been reported in these species for this region.

## MATERIALS AND METHODS

We collected museum-quality amphibian specimen vouchers in south-central Pennsylvania as part of a methodical and long term effort to document the current herpetofauna of the state. Our collection was approved by the proper state regulating agency; Pennsylvania Fish and Boat Commission (Scientific Collecting Permit 199 type 1). We also followed museum collecting standard protocols (PISANI & VILLA, 1974) adopted by the State Museum

of Pennsylvania (Harrisburg, Pennsylvania). This particular series of vouchers helped us determine reproductive and morphometric parameters of the poorly studied amphibians of the south-central region of the state. Breeding individuals were collected by hand at night on 28 February and on 6, 10, and 17 March 2011, from a series of interconnected temporary ponds of approximately 0.2 ha in combined surface area in the proximity of Boiling Springs, Cumberland County, Pennsylvania. During this period, the numbers of adults of both species were comparable but the sex ratios reflect temporal availability, with lower numbers of females in later sampling dates. All specimens were euthanized and immediately fixed in formalin after capture, and were stored in 70% ethyl alcohol in the section of Zoology and Botany of the State Museum of Pennsylvania. Prior to dissection, the body size of each specimen was measured from the tip of the snout to the caudal end of the vent, snout-vent length (SVL), to 0.1 mm using calipers. Presence of swollen cloacal lips and enlarged testes were the criteria used to determine sexual maturity in males. Presence of enlarged oviducts or mature ova was the criterion used to determine sexual maturity in females. Mature ova were removed from each female, patted dry with a paper towel, and weighed to the nearest 0.1 g using an electronic scale. To determine the body mass of the females with clutches removed, each specimen was also patted dry and weighed to the nearest 0.1 g. Because food was absent in these gravid females, stomach contents did not need to be removed from the digestive tracts.

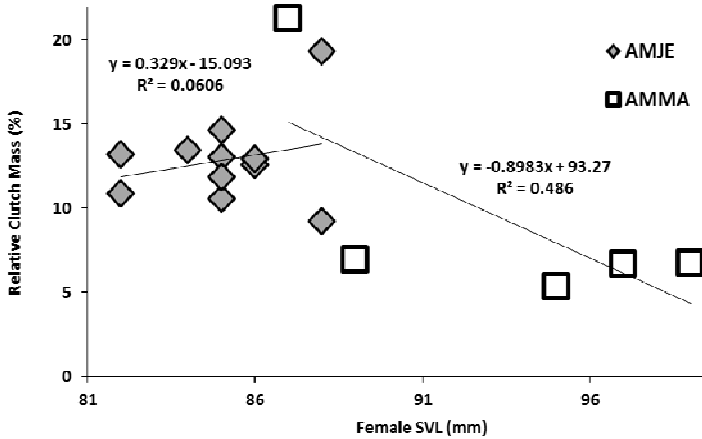
Clutch size was estimated by counting all the mature ova in each female. A measure of female relative reproductive effort was determined by calculating relative clutch mass (RCM) using the following formula:  $RCM = [\text{clutch mass} / (\text{clutch mass} + \text{body mass without clutch})] \times 100$ .

The diameters of 10 ova from each clutch were measured to 0.1 mm using an ocular micrometer in a dissecting microscope. The mean and the maximum values of the egg diameters were used in subsequent comparisons with body size and clutch size. Two-sample, two-tailed, with unequal variance t-tests were used to compare sample means, ANOVA F-tests were used to compare egg diameter and clutch size variances between species, and a Pearson Correlation was used to test for statistical significance of related variables. Data in our analyses met parametric assumptions and therefore did not require log-transformation or any other mathematical adjustment (ZAR, 1996). All statistics and quantitative graphics were produced and calculated using Excel 2007 (Microsoft Inc.), and statistical significance was recognized at a p-value of at least 0.05.

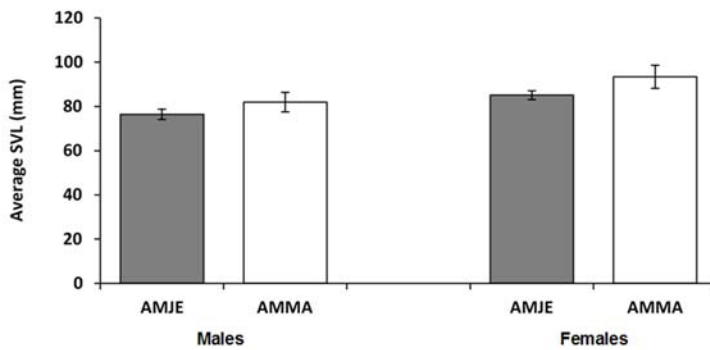
## RESULTS

### *Ambystoma jeffersonianum*

The mean SVL ( $\pm$  SD) of males ( $76.0 \pm 2.4$  mm; range = 72-82; N = 14) was significantly smaller ( $t_{23} = -9.643$ ;  $P < 0.001$ ) than that of females ( $85.1 \pm 2.0$  mm; range = 82-88; N = 11). Based on mean adult body size, male:female body size dimorphism was 0.89:1.00. Mean clutch size ( $\pm$  SD) was  $169.6 \pm 2.4$  eggs (range = 127-198; N = 11), and clutch size was not significantly asso-



**Figure 3:** Relative clutch mass (RCM) relationship to female snout vent length (SVL) between the Jefferson Salamander (*Ambystoma jeffersonianum*) (AMJE) (N = 11) and the Spotted Salamander (*A. maculatum*) (AMMA) (N = 5) from Cumberland County, south central Pennsylvania. Best-fit linear relationship and correlation coefficient expressed for both species.



**Figure 4:** Average ( $\pm$  SD) snout vent length (SVL) by sex in Jefferson Salamanders (*Ambystoma jeffersonianum*) (AMJE) (N = 14 males and 11 females) and Spotted Salamanders (*A. maculatum*) (AMMA) (N = 42 males and 5 females) from Cumberland County, south central Pennsylvania.

ciated with female body size ( $r = 0.18$ ;  $P = 0.032$ ). Mean RCM ( $\pm$  SD) was  $12.9 \pm 2.7\%$  (range = 9.2-19.4; N = 11). No significant relationship was detected between RCM and female body size ( $r = 0.24$ ;  $P = 0.033$ ) (Fig. 3). Mean ovum size ( $\pm$  SD) was  $2.4 \pm 0.2$  mm (range = 2.0-2.9; N = 110). Neither mean nor maximum ovum size exhibited any significant relationship with either female body size (mean ovum size:  $r = 0.55$ ;  $P = 0.07$ ; maximum ovum size:  $r = 0.51$ ;  $P = 0.10$ ) or clutch size (mean ovum size:  $r = 0.10$ ;  $P = 0.86$ ; maximum ovum size:  $r = 0.15$ ;  $P = 0.62$ ).

### ***Ambystoma maculatum***

The mean SVL ( $\pm$  SD) of males ( $82.0 \pm$

4.4 mm; range = 72-91; N = 42) was significantly smaller ( $t_{45} = -5.438$ ;  $P < 0.001$ ) than that of females ( $93.4 \pm 5.2$  mm; range = 87-99; N = 5). Based on mean adult body size, the male:female body size dimorphism was 0.88:1.00. Mean clutch size ( $\pm$  SD) was  $130.8 \pm 8.0$  eggs (range = 123-143; N = 5), and clutch size was not significantly associated with female body size ( $r = 0.78$ ,  $P = 0.11$ ). Mean RCM ( $\pm$  SD) was  $9.4 \pm 6.8\%$  (range = 5.3-21.3; N = 11). No significant relationship was detected between RCM and female body size ( $r = -0.69$ ;  $p = 0.033$ ) (Fig. 3). Mean ovum size ( $\pm$  SD) was  $2.6 \pm 0.2$  mm (range = 1.8-3.0; N = 50). Neither mean nor maximum ovum size exhibited any significant relationship with either

female body size (mean ovum size:  $r = -0.12$ ;  $P = 0.85$ ; maximum ovum size:  $r = -0.01$ ;  $P = 0.98$ ) or clutch size (mean ovum size:  $r = 0.31$ ;  $P = 0.60$ ; maximum ovum size:  $r = 0.33$ ;  $P = 0.59$ ).

### *Interspecific comparisons*

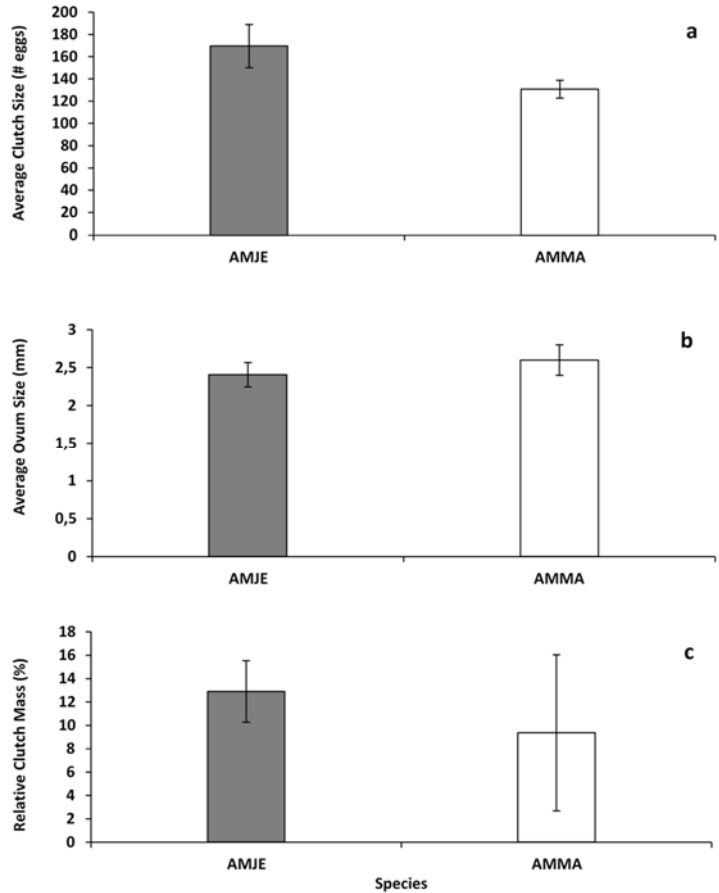
Among males of both species, adult body size of the Jefferson Salamander was less variable and smaller with respect to mean ( $t_{41} = -5.923$ ;  $P < 0.001$ ) than that of the Spotted Salamander (Fig. 4). Similarly, among females of both species, adult body size of the Jefferson Salamander was less variable and smaller with respect to mean ( $t_5 = -3.476$ ;  $P = 0.02$ ) than that of the Spotted Salamander (Fig. 4). Although smaller in mean adult body size, the Jefferson Salamander produced clutches whose sizes were greater in variability ( $F_{4,11} = 5.898$ ;  $P = 0.05$ ) and larger in size ( $t_{14} = 5.636$ ;  $P < 0.001$ ) than did the Spotted Salamander (Fig. 5a). Conversely, the Spotted Salamander produced eggs whose diameters were greater in variability ( $F_{4,11} = 0.632$ ;  $P = 0.03$ ) and mean ( $t_{78} = -6.003$ ;  $P < 0.001$ ) than were those of the Jefferson Salamander (Fig. 5b). The average RCM was nearly 37% greater in the Jefferson Salamander than that of the Spotted Salamander and this difference was not statistically significant ( $t_{25} = -0.812$ ;  $P = 0.21$ ) (Fig. 5c).

### DISCUSSION

We collected a logistically feasible and somewhat representative assortment of Jefferson Salamanders (25 total: 14 males and 11 females) and Spotted Salamanders (47 total: 42 males and five females) at a single location in south Cumberland County, south-central Pennsylvania. The

breeding sites were surveyed over a period of 17 days during which we identified large aggregations of ambystomatid salamanders, exclusively Jefferson and Spotted Salamanders, as well as Wood Frogs, *Lithobates sylvaticus*, across the different vernal pools. This precise assemblage of amphibian species, relatively early in the spring season, is typical in the northeastern USA (HULSE *et al.*, 2001). Although our purpose was not to determine population sizes or sex ratios, cursory examination of the sites showed that the Spotted Salamander was significantly more abundant than the Jefferson Salamander and characteristic dominance of males over females within each of the species (BRODMAN, 2005; SAVAGE & ZAMUDIO, 2005). The differential ratio of males to females was much more biased in the Spotted Salamander in which the four-day, near eight-hour search produced only four females.

Within our specific sampling, the body sizes and the direction of body size dimorphism found in both species at our site in south-central Pennsylvania typified findings from western populations (MINTON, 2001) and elsewhere in the northeast (KLEMENS, 1993). Our findings are also consistent with those generally reported for Pennsylvania (HULSE *et al.*, 2001). For both sexes, the Spotted Salamander was the largest species, and females were the largest sex in both species (KLEMENS, 1993; HULSE *et al.*, 2001; MINTON, 2001). These patterns of size sexual dimorphisms have been explained in the literature as the result of sexual selection (DARWIN, 1874) following an evolutionary strategy to maximize reproductive output via larger female coelomic cavity (KAPLAN & SALTHER, 1979).



**Figure 5:** Average ( $\pm$  SD) clutch size (a), average ovum size (b), and average reproductive effort measured as relative clutch mass (RCM) (c) from the Jefferson Salamander (*Ambystoma jeffersonianum*) (AMJE) (N = 11) and the Spotted Salamander (*A. maculatum*) (AMMA) (N = 5) from Cumberland County, south central Pennsylvania.

In general, female fecundity increases with increasing body size more rapidly than does male reproductive success, thereby selecting for larger body size in females than in males (TRIVERS, 1972; CRUMP, 1974).

In our study, all females captured contained body cavities with fully mature ova and oviducts completely clean. These conditions indicated that no eggs had yet been laid upon collection and reflecting that our sampling took place nearly at the peak of breeding activity, rendering our data more representative. Fecundities, clutch size

estimates from our study, were mostly within the range of other studies in the Jefferson Salamander. For instance, to our west, means of 183 and 212 ovarian egg counts were reported for two Jefferson Salamander Ohio samples (UZZELL, 1964), and to our east a mean of 156.5 ovarian eggs was published from New York (BISHOP, 1941). For the Spotted Salamander, however, our sample, even if limited, clearly shows smaller clutch sizes contrasting the few known reports from other regions. For example, clutches of the Spotted Salamander ranged from 144 to

370 eggs in Connecticut (WOODWARD, 1982), reached up to 300 eggs in Louisiana (DUNDEE & ROSSMAN, 1989), showed a mean of 224 ovarian eggs that ranged 92-328 in Massachusetts (SHOOP, 1974), averaged 207 ovarian eggs in Virginia (IRELAND, 1989), and showed a mean of 172 ovarian eggs in Michigan (WILBUR, 1977). More and broader sampling of Spotted Salamanders at our region in the near future will likely shed light over these differences.

Studies in patterns of fecundity point to female body size as the main determining factor on clutch size variation (VITT & SEIGEL, 1985; REISS 1989). When historically examining salamander reproductive parameter relationships, clutch size and SVL positively correlated at one of two sites examined in Connecticut (WOODWARD, 1982). We leave open the possibility that the absence of a significant positive relationship in our sample could have been an artifact of small sample size of the Spotted Salamander. The Jefferson Salamander, likewise, did not exhibit a significant relationship between clutch size and female body size. Neither species exhibited any significant relationship between maximum ovum size and female body size, or between RCM and female body size suggesting that these clutch characteristics were affected by variables other than the body size of the female, such as female age or energy-linked physiological condition. Maximum ovum size was also independent of clutch size. Intraspecifically, body size is often but not always a predictor of clutch size (KAPLAN & SALTHER, 1979), and in both salamander species of our study, no body size advantage was associated with

clutch size. In light of small sample sizes, we cannot rule out an artifact of sampling as responsible for the non-significant relationship. A conservative evolutionary strategy associated with ovum size could explain an overall absence of relationship between ovum size and female body size in ambystomatid salamanders (KAPLAN & SALTHER, 1979), a finding shared by both species in our study. Predictably, an increase in clutch size would be accompanied by a decrease in ovum size unless a change also occurred in body size (KAPLAN & SALTHER, 1979). In our samples, however, clutch size and ovum size were also unrelated statistically. While this might be also the result of the small sample size in the case of the Jefferson Salamander, it is unlikely so for the Spotted Salamander. The later might be more a clear reflection of the understandable pliability of these reproductive relationships.

When speculating more in depth on the relationship between body size and clutch size, elsewhere in its range, the Spotted Salamander was found to be less fecund than expected in a body size model (KAPLAN & SALTHER, 1979). At our site, the larger clutch size of smaller eggs produced by the Jefferson Salamander, the smaller-bodied of the two species, was noteworthy and intriguing to us because it provided this species with a potential advantage even before hatching by sheer fecundity at an expense of hatchling body size. Our study therefore suggested that the evolutionary strategy was for the Jefferson Salamander, with larger clutch masses, to overwhelm the site with predaceous young in advance of its congeneric competitor and prey species whose response



was to produce fewer but larger eggs and presumably larger young. These larger young are notorious predators on their congeners (BRODMAN, 2005; SAVAGE & ZAMUDIO, 2005). The extent to which these characteristics are variable annually or in allopatric situations is unknown but would explain the importance of clutch characteristics in co-existence of the Spotted Salamander with its more fecund predator, the Jefferson Salamander, at the larval stage.

The present dataset adds an interesting mix of reproductive information that raises more questions about the difficult mosaic of characteristics determining the complex ontogenetic strategies, and the resulting biological fitness, of these two species. A wide array of reproductive characteristics such as the time of egg deposition (WILLIAMS, 1973; HILLIS, 1977; MINTON, 2001), the number of eggs produced (BISHOP, 1941; UZZELL, 1964; SHOOP, 1974; WILSON, 1976; BRODMAN, 1995; PETRANKA, 1998), the size of those eggs (BRODMAN, 2005; SAVAGE & ZAMUDIO, 2005), the speed of development (WORTHINGTON, 1968; CORTWRIGHT, 1988; TALENTINO & LANDRE, 1991; MINTON, 2001), the size of the hatchlings (BRODMAN, 2005; SAVAGE & ZAMUDIO, 2005), as well as the morphological characteristics of these predatory larvae (MOTT & MARET, 2011), all interact to produce ultimately an ecological advantage that will result in long term evolutionary overall superiority.

Our study underscores also the importance of life history studies such as ours, which determined fecundity and egg sizes directly from ovarian counts rather than from dispersed clutches in the field.

We believe that with greater sample sizes, we might answer this multifaceted, as well as puzzling, competitive game between the Jefferson and the Spotted Salamanders. Furthermore, this information will likely help us understand the complex and dynamic relationships between these two ambystomatid salamanders, not just in south-central Pennsylvania but across their full range. We advocate that studies like ours, in conjunction with long term population monitoring, are crucial to determine reliably present amphibian demographic trends. These sorts of data will also allow us to more effectively devise future strategies for the conservation of species of amphibians around the world.

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