The Relation between Ovum Variability and Larval Growth in *Ambystoma tigrinum*, Amphibia: Urodela

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Introduction

In three species of ambystomatid salamanders, including *A. tigrinum*, Kaplan (4) reported that larger eggs result in hatchlings that reach the feeding stage earlier than hatchlings from smaller eggs. Subsequently, the absolute difference in size between the larvae resulting from large eggs and those from smaller eggs becomes amplified with time (4). Larger larvae may be able to metamorphose more quickly, and this might be adaptive in an ephemeral pond environment. Thus, the size of eggs that a female produces may have implications for her reproducive fitness (4, 6, 11). Walls and Altig (11), however, found that the relation between egg size, hatchling length and larval growth held for some species of *Ambystoma* but not for others. Also, although larger eggs produce larger larvae that reach the feeding stage earlier than smaller larvae in the newt *Taricha torosa*, Kaplan (5) reported that larvae from large eggs metamorphose in less time and at a larger size than those from small eggs only in a food unlimited situation. In a food limited situation, larvae from larger eggs still were larger at metamorphosis but the time to metamorphosis was greater than that of larvae from smaller eggs. Finally, Nussbaum (7) noted that the ranges of egg size across populations of ambystomatids are small and suggested that factors other than egg size, both genetic and ecological, may be more important in determining the length of the embryonic period.

We studied a population of *A. tigrinum* that breeds in a temporary pond in northern Indiana. Previous studies on this population have shown that hatching of eggs is nearly synchronous, but great differences in subsequent growth occur among the larvae (6, 9). Our purpose was to see if the range of larval size could be explained by concordant variation in ovum size.

Materials and Methods

Snout-vent length (svl) was measured from tip of the snout to posterior end of the vent with calipers to the nearest 0.5 mm in living specimens and nearest 0.1 mm in preserved specimens. Statistical tests followed Sokal and Rohlf (10).

The study pond was located in St. Joseph County, Indiana. This locale and its breeding population of *Ambystoma tigrinum* were described in detail by Sever and Dineen (9). Briefly, the pond is formed by melting snow and rainfall in winter, and at maximum size, the pond is 0.25 ha and 90 cm deep. The pond dries completely each summer. *Ambystoma tigrinum* is the only salamander using the pond, but *Bufo americanus* breeds in the pond and two other anurans, *Pseudacris triseriata* and *Hyla versicolor* probably do.

In 1985, five females 9.7-12.4 cm svl ($\bar{x}$ = 11.0 mm, S.D. = 1.06) were collected from the study pond following mating but prior to oviposition as determined by presence of a spermatophore cap in the cloacal orifice. These females oviposited in the laboratory, and clutch size was 344-543 ($\bar{x}$ = 450.8, S.D. = 88.2). Following preservation of the eggs in 10% formaldehyde, ova diamters were measured to the nearest 0.01 mm using an ocular micrometer in a dissecting microscope. Mean egg diameters were 1.58-1.68 mm, and a one-way analysis of variance showed significant differences ($p < 0.5$, F = 331.8) in mean ovum diameter among females. From this preliminary study, we determined that measuring 25 eggs from a single female was sufficient to be within 0.03 mm of the true mean ovum diameter of the entire clutch at a 95% confidence level.

On 12-14 March, 1986, 10 gravid female *A. tigrinum* 9.5-11.5 cm svl ($\bar{x}$ = 10.5, SD = 0.77) were collected from the study pond. Some females had spermatophore caps visible in the cloacal orifice, but others did not, and these latter individuals may have oviposited some eggs in the pond prior to their collection. The females were placed in tanks containing twigs for attachment of egg masses. Oviposition occurred within 2 days of collection for all females. Clutches contained 228-815 eggs ($\bar{x}$ = 448.8, S.D. = 197.2). From each clutch, 25 eggs were randomly selected and measured to the nearest 0.02 mm.

On 17 March, the clutches were taken to the pond and placed in containers that were distributed evenly around the pond and submerged in 60 cm of water. The containers were plastic buckets 36 cm in length × 29 cm in widest diameter with openings $\sim$20 cm in diameter cut in the bottom and in the lid. Over the openings wire mesh was stapled. The mesh size, $\sim$1.5 mm square, was large enough to allow passage of small invertebrate prey items (9) but small enough to prevent passage of hatchling salamander larvae.

The larvae in the 10 buckets hatched by 11 April. On that date, the number of larvae that had survived to hatching in each clutch was noted. Larvae were anesthetized in an ether solution (1:9 water), and 25 larvae were randomly chosen from those in each bucket and measured. After recovery from anesthesia, the larvae were returned to their buckets. No immediate mortality from the anesthetic was noted.

All living larvae in each bucket subsequently were measured on 25 April and 9 May. Measurement of larvae was discontinued after 9 May due to high mortality, including complete mortality in two buckets.

In addition, a minimum of 25 free swimming larvae were collected from the pond on 11 April, 25 April, 9 May and at 14-18 day intervals until the pond dried completely on 22 August. These larvae were measured after preservation in 10% formaldehyde.

Results

Mortality of Eggs and Larvae

Mortality data for the eggs and larvae in each container are given in Table 1. Overall, mortality from oviposition to hatching was 63.5-98.1% ($\bar{x}$ = 87.3%, SD = 9.93). Cumulative percent mortality between oviposition and 25 April was 90.7-99.6% ($\bar{x}$ = 95.0%,

TABLE 1. Cumulative percent mortality (CPM) among eggs and larvae reared in containers in the study pond.

Bucket	17 March	11 April		25 April		9 May	
	N	N	CPM	N	CPM	N	CPM
1	433	51	88.2	23	94.7	5	98.8
2	815	157	80.7	76	90.7	9	98.9
3	265	36	86.4	19	92.8	14	94.7
4	604	82	86.4	44	92.7	14	97.7
5	695	44	93.7	28	96.0	9	98.7
6	318	43	86.5	21	93.4	11	96.6
7	359	131	63.5	28	92.2	5	96.2
8	297	23	92.3	3	99.0	1	99.7
9	464	9	98.1	4	99.1	0	100
10	228	7	96.9	1	99.6	0	100

SD = 3.23) and between oviposition and 9 May was 94.7-100% ($\bar{x}$ = 98.1, SD = 1.79). Following hatching, mortality may have been influenced by cannibalism. One larvae in a container was observed engulfing another on 25 April.

Variation in Size of Ova and Larvae

Lengths of the larvae in the buckets were compared with those of free-swimming larvae collected on the same dates that the caged larvae were measured. Since the free-swimming larvae were undoubtedly of multiple parentage, a group mean for them was compared with a group mean for all the larvae measured from the buckets on a given date. No significant difference ($p > 0.05$) occurred between mean larval length for the caged and free-swimming larvae on 11 April, but differences between the means were significant ($p < 0.05$) for larvae from 25 April and 9 May (Table 2). The mean for the free-swimming larvae ws larger than that for the larvae in buckets on 25 April and 9 May.

TABLE 2. Differences in snout-vent length between larvae in the buckets (Caged) and free-swimming larvae (Free). The F ratio for the 11 April larvae was not significant ($p > 0.05$) while those for 15 April and 9 May were significant ($p < 0.05$). Measurements are in mm.

Date	Caged				Free				F
	N	Range	Mean	SD	N	Range	Mean	SD	
11 April	222	5.0- 9.0	7.59	0.80	25	6.0- 9.0	7.72	1.02	0.55
25 April	248	7.0-15.0	10.69	1.45	23	9.0-14.0	11.56	1.47	7.72
9 May	68	11.5-32.0	18.77	4.01	23	19.0-32.0	24.24	3.81	32.71

The comparisons in Table 2 were made between larvae from the buckets measured alive while anesthetized and free-swimming larvae measured after preservation in 10% formaldehyde. To check the effects of preservation on the measurements, the free-swimming larvae collected 9 May were measured alive while anesthetized and remeasured after preservation. Measurements of the living specimens were 19-34 mm svl ($\bar{x}$ = 25.5 mm, SD = 4.07) as opposed to 19-32 mm svl ($\bar{x}$ = 24.2 mm, SD = 3.81) for preserved larvae, not a significant difference at $p < 0.05$ (F = 1.26).

One-way analyses of variance were conducted to test the hypotheses that no variation existed among females in the size of their eggs at oviposition or their larvae on any of the dates when the larvae from the buckets were measured. Because complete mortality occurred in two buckets by 9 May, only the eight buckets that still contained larvae on 9 May were used in the analyses. The null hypotheses were rejected. Variance was significant at $p < 0.05$ for ovum size (F = 15.70), 11 April larval lengths (F = 7.50), 25 April larval lengths (F = 3.56) and 9 May larval lengths (F = 2.79).

Hochberg's GT-2 *a posteriori* test was used to see how the means analyzed contributed to rejection of the null hypotheses. This test found subsets of means within which differences were not significant at $p > 0.05$. Mean egg sizes were 2.09-2.32 mm, and five overlapping subsets of means were found. Three subsets of means were found for 11 April larval lengths (6.88-7.30 mm, 7.30-7.92 mm, and 7.64-8.00 mm). Significant variances on 25 April and 9 May were due to differences between the lowest and the highest means. Mean larval lengths on 25 April were 9.76-12.83 mm with subsets of 9.76-11.02 mm and 10.55-12.83 mm. For 9 May, the range was 14.90-23.00 mm with subsets of 14.90-20.50 mm and 16.68-23.00 mm.

Thus, larvae from seven of the buckets on 25 April and 9 May did not differ in mean svl length from one another. The buckets containing the largest and smallest larvae were not the same on these two dates. Bucket 7 actually changed from the container having the smallest larvae on 25 April to containing the largest larvae on 9 May.

The maximum range in larval size in the buckets after hatching was 2.5 mm (6.5-9.0 mm) and the minimum was 1.5 mm (6.0-7.5 mm). On 25 April, the maximum range in larval size was 7.0 mm (8.0-15.0 mm), and the minimum was 4.0 mm (9.0-13.0 mm) excluding bucket 8, in which just three larvae (12.0-13.5 mm) remained. In the 5 buckets with N $\geq$ 9 larvae on 9 May, the maximum range of larval sizes was 17.0 mm (15.0-32.0 mm), and the minimum range was 6.5 mm (13.5-20.0 mm).

Variation in larval size among free-swimming larvae also was small at hatching but increased markedly by 9 May (Figure 1). Thereafter, variation in svl of free-swimming larvae remained high with the greatest range, 26-58 mm, occurring on 11 July.

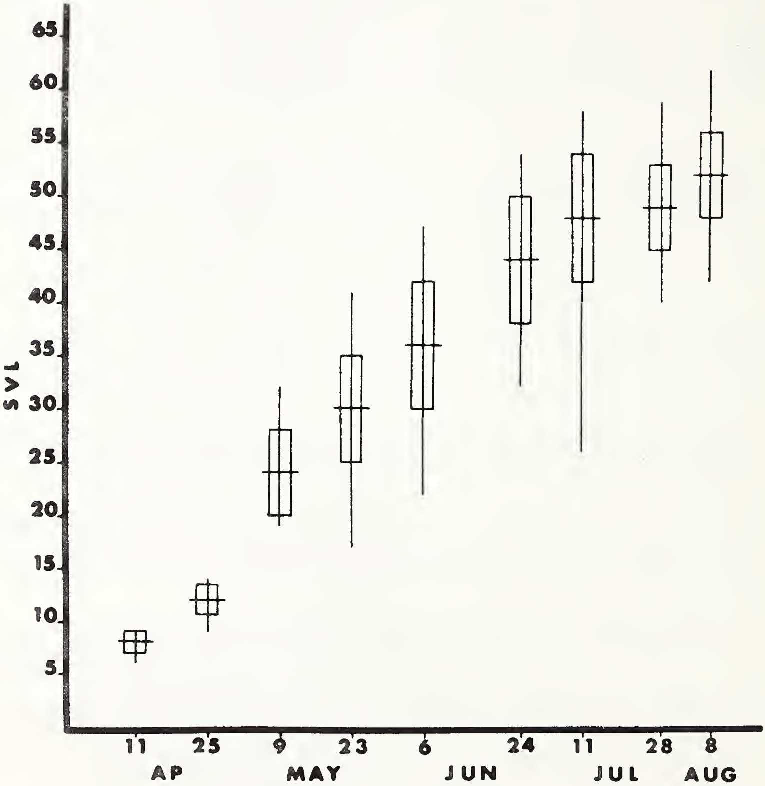

FIGURE 1. Growth of free-swimming larvae collected during the summer of 1986. The vertical line is the range in snout-vent length (svl); the horizontal line is the mean; and the box above and below the mean is one standard deviation.

Concordance of Ovum Size and Larval Growth

Kendall's coefiicient of concordance was used to test the null hypothesis that no relationship exists among the rank order of mean egg diameters and larval sizes in the sequential sampling data from the eight buckets used in the analyses of variance. The hypothesis was accepted as concordance of means was not significant at $p > 0.05$ ($W = 0.24$, $\chi^2 = 6.74$). Thus, the variation in larval size could not be attributed to a consistent pattern of variation in egg or larval size observed in the samples.

Discussion

Survivorship and Growth

Couture and Sever (2) followed mortality in clutches of *A. tigrinum* in natural situations in the study pond and reported 74.3% cummulative mortality from cleavage to neurla. This is less than the mortality we found in the buckets between oviposition and hatching (87.3%), but our results and those of Couture and Sever cannot be compared statistically.

Likewise, the significance of the mortality we found in the buckets through 9 May is unclear, as no comparable data on natural mortality during this period are available for free-swimming larvae in the study pond. Anderson *et al.* (1), in a population of *A. tigrinum* in New Jersey, found that 3.3% of the eggs deposited survived to metamorphosis. The relation of larval density to survivorship and growth in natural and artificial situations is complex (8, 12). Generally, higher survivorship to metamorphosis has been noted in amphibians in field enclosures versus natural habitats (8).

Growth of larvae may be related to density, with larvae growing slower under crowded conditions (8). In this regard our data indicate that at hatching larvae in the buckets were not different in mean svl from free-swimming larvae in the study pond, but that on our last two sample dates, free-swimming larvae were significantly larger than those in the buckets. Either we were not efficiently sampling smaller free-swimming larvae on 25 April and 9 May, or else the growth of free-swimming larvae was greater than that of larvae in the buckets. In the latter case, the presumed higher density of larvae in the buckets than in the pond may have affected their growth (8, 12).

Ovum Size and Larval Growth

Our results for the larvae in the buckets may not reflect the situation under natural conditions due to the influence of larval density and prey availability on growth of larvae (5, 8, 12). In particular, Kaplan's (5) report that larvae of *T. torosa* from large eggs grow faster than those of larvae from small eggs only in food unlimited situations may be relevant.

We do not know, however, whether the density of larval *A. tigrinum* and prey items in our enclosures was different from that for free-swimming larvae, or if density factors affect the growth of larval *A. tigrinum* like they influence growth of larval *T. torosa.* Indeed, the density of larval *A. tigrinum* in the enclosures may actually increase the growth rate of larger larvae since the larger larvae may find the smaller ones to be an abundant and concentrated source of prey. The cannibalistic nature of larval *A. tigrinum* is well-known (3, 6), and we observed cannibalism among larvae in the buckets during the study. In contrast, Sever and Dineen (9) did not find cannibalism occurring among free-swimming larvae in the study pond based upon an examination of stomach contents.

In bucket 7 on 25 April, 28 larvae occurred, and the mean for larvae in this bucket was the smallest of that for the eight buckets on this date. On 9 May, however, only 5 larvae were present in bucket 7, but the mean for these larvae was the largest of that for the buckets. Could cannibalism have been involved? If so, density of larvae increased the growth of those larvae large enough to engulf their siblings. Our laboratory and field observations indicate that the difference in size necessary for cannibalism to occur is small, although this has not been quantified.

Obviously, more work is needed before the dynamics of larval growth of *A. triginum* are elucidated in our study pond. Our reuslts demonstrate that: (1) a difference in mean egg size does occur among female *A. tigrinum*; (2) variability occurs in growth rates among the offspring of individual females; and (3) the variability in larval growth cannot be explained simply in terms of ovum size. Thus, although larval growth may be involved, the selective pressure for the number and size of eggs produced by female *A. tigrinum* in the study pond may include other factors as well.

Acknowledgments

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