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INTRAPOPULATIONAL VARIABILITY IN EGG SIZE: ITS ALLOMETRIC AND ENERGETIC RELATIONSHIP TO BODY SIZE IN THREE SPECIES OF EASTERN AMBYSTOMA

BY

ROBERT H. KAPLAN

A dissertation submitted to the Graduate
faculty in Biology in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy, The City University
of New York

1978

Robert Kaplan

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

March 31, 1978	
Date	Chairman of Examining Committee Professor S. Salthe
April 18, 1978	Louis G. Moriber
Date	Executive Officer Professor Louis G. Moriber
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INTRODUCTION

The key to the persistence of populations despite changing environments and natural selection, lies in differences among individuals. If the characteristics enabling an organism to produce an adequate number of viable offspring (a reproductive strategy) are being considered, then at any given time it follows that a continuum of successful strategies (not necessarily equally successful during any short time span such as a single breeding period in an iteroparous species) must exist even within one population. An outcome of this variability is the diversification of reproductive strategies through evolutionary time coinciding with speciation events.

While the above argument is implicit in reproductive strategy theory, little attention has been directed towards the actual quantification of intrapopulational variability of traits of this sort. Mean population values for characteristics such as age at first reproduction, breeding frequency, age-specific reproductive effort and survivorship are difficult enough to establish; the determination of variance for such characters is rarely feasible. Variance, however, can be determined for the more static characteristics of reproductive strategies such as body size and egg number. In this work these characteristics are combined with another static character (ovum size) resulting in a fourth character, total clutch volume, also becoming available.

A complete understanding of these characteristics alone is certainly not sufficient to elucidate an entire reproductive strategy. But an analysis of the magnitude and ramifications of intrapopulational variability in these characteristics will shed light on the degree of flexibility (or sloppiness) that can be tolerated by a population. The environmental determinants (selective pressures) that necessitate such flexibility can subsequently be ascertained and suggest insights concerning other features of the total strategy.

No single philosophical approach overly influenced the organic development of this work. Besides the general theoretical approach that has been developed to clarify our understanding of life-history strategies (see Pianka, 1976 and Stearns, 1976 for review), the heuristically successful model of Smith and Fretwell (1974) which relates parental fitness to parental investment per offspring and offspring fitness further treated by Brockelman (1975) and Wilbur (1977) , strongly influenced the way in which the data presented here were collected and analyzed. The more empirical approaches of Tilley (1968), Salthe (1969), Tinkle (1969) and Wilbur and Collins (1973) were also highly influential in the present study.

MATERIALS AND METHODS

The emphasis of the methodology in this study was to associate females with their respective offspring. This approach has been used previously in numerous analyses of clutch size - body size relationships, but very little attention has been given to ovum size due to the difficulty in obtaining such data (but see Wilbur, 1977). By emphasizing individual variation, body sizes of specific females are related directly to clutch parameters such as clutch size, ovum size and clutch volume. Subsequently, the influence of female-specific ovum size variability on potentially important embryonic variables such as developmental rate, size, and stage of larvae at hatching and feeding, the energetic cost of development, the efficiency of development, respiratory rate, and post feeding growth, are ascertained.

THE ANIMALS AND THEIR HABITATS

Five populations of three salamander species of the genus *Ambystoma* were studied during their breeding periods from 1974 through 1976. *Ambystoma tigrinum*, *A. maculatum* and *A. opacum* were selected because they cover a wide range of body sizes, are taxonomically related, and may, with reservation regarding *A. opacum*, (see below), be regarded as exhibiting the same reproductive mode.

Ambystoma t. tigrinum is one of the largest terrestrial salamanders in the world (plate 1). The population studied is located near Heislerville, Cumberland County, in the extreme southern part of New Jersey and is at the northeastern edge of the species' range (Gehlbach, 1967). The pond is one of a series of abandoned gravel pits, located

Plate 1. _Ambystoma_ _t_. _tigrinum_ from Heislerville, Cumberland County, New Jersey.

in a flat region surrounded by reeds and shrubs (plate 2). The species in this part of its range is a winter breeder, with an extended breeding season. During the course of this study eggs were found in early stages of development (pregastrula) as early as December 18 and continued to be found on rainy, "warm" nights (above 4° C) as late as March 2. The water temperature in which eggs were deposited and in which most embryos developed averaged approximately 7.6°C with a range from 3.2° - 11.1° C. Even when ice was several inches thick (which occurs sporadically throughout the winter), the water temperature in which the embryos were found did not drop below 3.2°C. The pH was always between 6.5 and 7.1. Females deposit eggs in small masses (an average of 52 eggs per mass) attached to vegetation under water. They were captured either while migrating to the ponds at night or by being netted within the pond on rainy nights. Few females came to the pond at one time, where they were received by waiting males. The ponds were visited on most rainy nights between the middle of December and early March beginning in December 1974 and ending in March 1976. Thus, two breeding seasons were followed intensively. Most animals in the pond were marked and newly arriving females could be readily distinguished. On several occasions females were found several days after a rain. These generally had many fewer eggs than expected and were not used in subsequent analyses. Hassinger _et_ _al_. (1970) gave a more detailed account of the physiography, climatic conditions and fauna found at this study site. Most animals were taken from one pond known as "megamiddle" (see also Anderson _et_ _al_., 1971).

The population of _Ambystoma_ _maculatum_ (plate 3) that I considered is located near Springdale, Sussex County, in northwestern New Jersey,

Plate 2. Ambystoma t. tigrinum habitat. ("megamiddle" of Anderson et al., 1971).

Plate 3. Ambystoma maculatum from Springdale, Sussex County, New Jersey.

well within the species' range (plate 4; Anderson, 1967a). The pond is located in one of the relatively rare limestone areas of the northeast. As a result the pH of the pond at the time of breeding was relatively constant at 7.5. The pond is surrounded by an oak-hickory-walnut deciduous forest in association with old fields. It is temporary, but at its maximum size in the early spring reaches a width of 70 m. and a length of 100 m. The depth is a maximum of 3 m. There is an extensive invertebrate and herpetofauna, the latter including the salamanders Notophthalmus viridescens, Eurycea longicauda and the ambystomatids jeffersonianum, platineum, opacum and maculatum. The pond is bordered by a thick stand of sapling red ash to which the maculatum attach their one or rarely, two egg masses (plate 4). During the course of this study, newly deposited eggs were observed as early as March 25 and as late as April 22. The breeding season is relatively short and is usually characterized by one large migration of females. The average water temperature at which eggs were deposited was approximately 10°C and ranged from 7.0°- 13.0°C. Gravid females were captured on land as they migrated to the ponds, either by hand or with the use of a drift fence with pitfall traps, constructed around a heavily traveled portion of the pond. Trips were made to the pond on most rainy occasions during the months of March and April in 1975 and 1976. A more detailed account of the natural history of this pond was given by Anderson and Martino (1966) who called it Springdale "A".

Ambystoma opacum is one of the two salamanders in the genus that deposits its eggs terrestrially (plate 5). It is the only member that remains with the eggs after they are deposited, presumably providing offspring with some sort of parental care (Kaplan and Crump, 1978).

Plate 4. Ambystoma maculatum habitat. (Springdale "A" of Anderson and Martino, 1966).

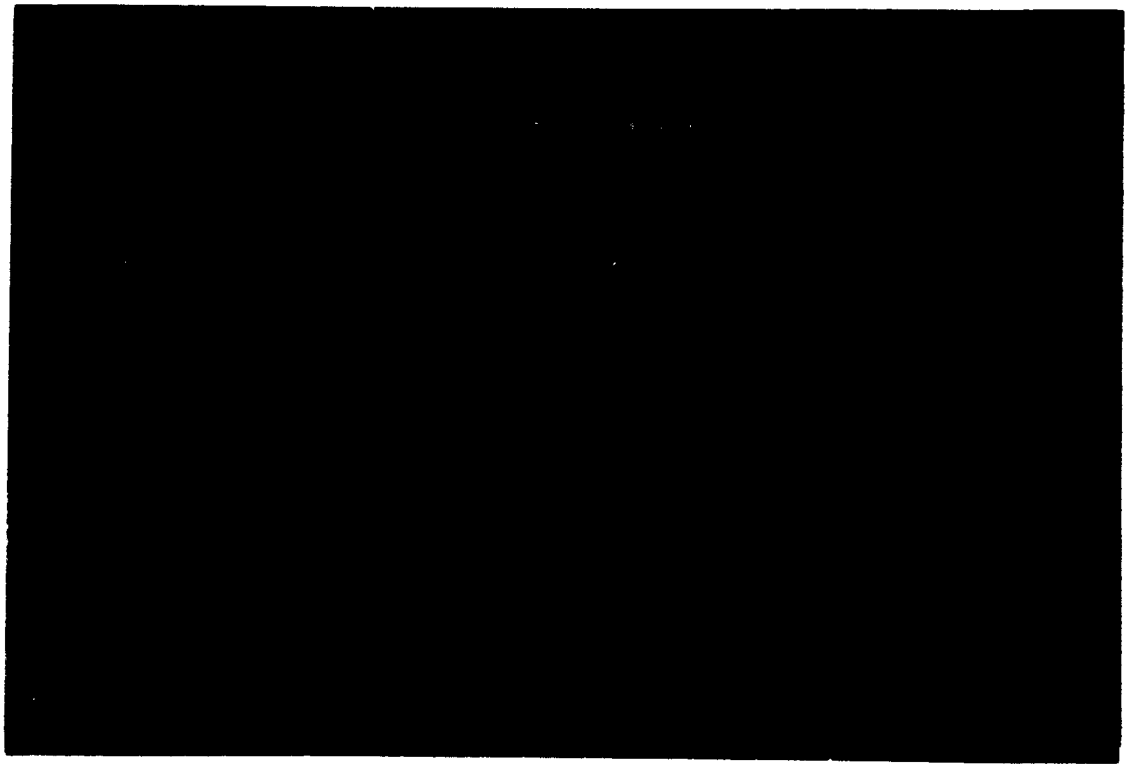


Plate 5. Ambystoma opacum brooding a clutch of eggs. (Photograph by Martha L. Crump).

Three populations of this species were studied: one from Swartswood, Sussex County, in northwestern New Jersey (hereafter referred to as A. opacum-N.J.); one from Tillman, Jasper County, in southern South Carolina (A. opacum-S.C.); and the other from Savannah, Chatham County, Georgia (A. opacum-Ga.). The New Jersey population is near the northern limit of the species range, while the two southern populations are near the southern end and more centrally located (Anderson, 1967b). Individuals of all populations deposit their eggs in one mass, under logs, rocks and leaf litter, in a dry pond bed. Deposition occurs during the fall before the winter rains which subsequently inundate the nests. The females then abandon the eggs and the typical Ambystoma type larvae hatch (Brandon, 1961).

The New Jersey population is found in the same limestone area as the A. maculatum population described above. The pond (=Peregino's pit) is surrounded by silver maple, sycamore and willows and is a steep-walled, triangularly-shaped quarry. It is 66.8 m. long and 31.7 m. wide when full. Often at the time of egg deposition, which occurs during a short period of time during mid-September, the pond is almost dry (plate 6a). Within even a several week period, rains can increase the depth to as much as 3.3 m. (plate 6b). At the time of egg deposition, soil temperatures are between 12° and 15°C and gradually diminish. (For a detailed analysis of the environment and population habits at this site see Graham, 1971).

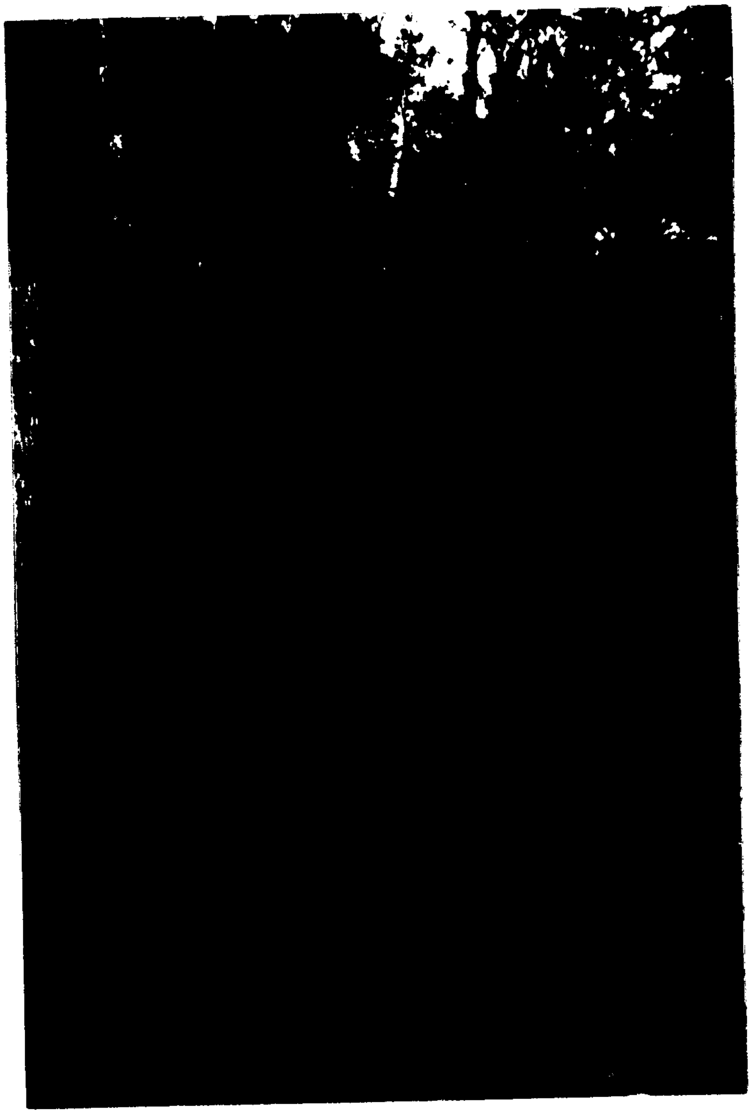
Anderson and Williamson (1973) presented evidence that the time of oviposition is temperature dependent. Females at the South Carolina and Georgia sites (plates 7 and 8) deposit eggs in the early part of November, when ground temperatures are similarly between 10° and 15°C.

Plate 6. a. Ambystoma opacum habitat (=Peregino's pit), Swartswood, Sussex County, New Jersey, at time of oviposition. (Clutches and attending females are scattered around the walls of the quarry).

b. Several weeks later.

Plate 7. Ambystoma opacum habitat, Tillman, Jasper County, South Carolina.

Plate 8. Ambystoma opacum habitat, Savannah, Chatham County, Georgia.



The populations studied are located near pine flatwoods on the eastern coastal plan. Both areas are dominated by bald cypress, sweet and black gums. Anderson and Williamson (1976) described the flora, fauna and other features of this temporarily dry environment.

Females attending their newly deposited eggs were collected by rolling logs, stones and scratching through leaf litter. Rainy weather was not found to be as reliable an indicator of breeding activity as it was in the other two species. Therefore, trips to the New Jersey site were made every two or three days beginning the first week in September and ending the first week in October from 1974 through 1976. One month of continuous field work beginning the last week in October through the end of November during the same three year period was undertaken at the South Carolina and Georgia sites.

FIELD METHODS

With the two aquatic breeding species, gravid females were captured (as described above) and placed in previously constructed breeding pens that were located in the ponds in about 0.3 - 0.6 m. of water. The cylindrical pens were made of 1.2 cm. mesh hardware cloth, with a diameter of approximately 0.6 m. and a height of approximately 1.0 m. (see plate 2). The pens were equipped with appropriate substrata (twigs) for egg deposition and contained 2 or 3 males. After all animals were put into the pens the top was tied together to prevent the animals from climbing out. They were left overnight or until the female deposited her clutch. Egg deposition usually took place after 1 - 3 nights. In this way each clutch could be associated with a specific female. Breeding pens were not necessary in the case of *Ambystoma opacum*, because females could be found attending their eggs. Females and eggs of all species were taken back to the laboratory for subsequent analysis.

LABORATORY METHODS

Body size

Initially females were sacrificed in order to determine if they had deposited their full complement of eggs. After it was found that all eggs were deposited (except in cases where females were interrupted during oviposition), females were anaesthetized with MS-222 or ethyl ether in order to take appropriate body size measurements and subsequently released into their own habitats. Length was measured from the tip of the snout to the posterior margin of the vent (SVL), and to the tip of the tail (TL). In addition an estimate of body volume was desired that would make the body size data comparable to those reported by Salthe (1969). Therefore, body volume was estimated by assuming the salamander to be a cylinder with a length extending from the gular fold (neck) to the posterior margin of the vent and with a diameter measured by the distance between the junction of the anterior limbs with the body on the ventral surface (axillary width). (See discussion for comments on this technique). It is felt that estimates of body volumes are useful when considering body size constraints associated with reproductive parameters. Wet weights (which are subject to water content variability) and dry weights include variability in head, limb and tail sizes which can obscure the variability in the functional size of the animal. (Tail size variability can be energetically important, but in the present context is ignored.)

Clutch size

All eggs were individually counted in order to determine clutch sizes. In the cases where females were interrupted during oviposition, non-deposited eggs were added onto the counts.

Ovum size

Ovum diameters were measured to the nearest 0.02 mm. using a dissecting microscope with an ocular micrometer. The diameters of the vitellus of 10 ova per clutch were then determined. It did not matter whether or not the egg capsules were removed, providing only that the egg with accompanying capsules was completely submerged during measurement.

Ovarian egg measurements are not considered reliable for fine intra-populational comparisons for several reasons: 1). All females may not be at similar stages of vitellogenesis at the time of measurement. 2). Ovarian ovum size measurement of preserved or even fresh material is made difficult due to the irregularities of the ova while they are attached to the ovarian wall. 3). Preserved material is subject to shrinkage and differential shrinkage probably exists depending on the stage of maturity of the ova. 4). Freshly dissected ovarian eggs swell rapidly (Løvtrup, 1960; Tuft, 1962); their size significantly increases during the time it takes to measure even ten eggs.

The measurement of recently deposited eggs is more appropriate but also has difficulties associated with it. 1). Populations must be continually monitored in order to get eggs prior to cleavage. 2). Embryos that are undergoing development, even though they maintain a round shape are subject to size changes (Løvtrup, 1960). In this study it was found that collecting eggs prior to first cleavage was not a very common experience, therefore it was necessary to work out the size increase that accompanies development up to the time that the embryo is no longer spherical, when measurement of size becomes complicated.

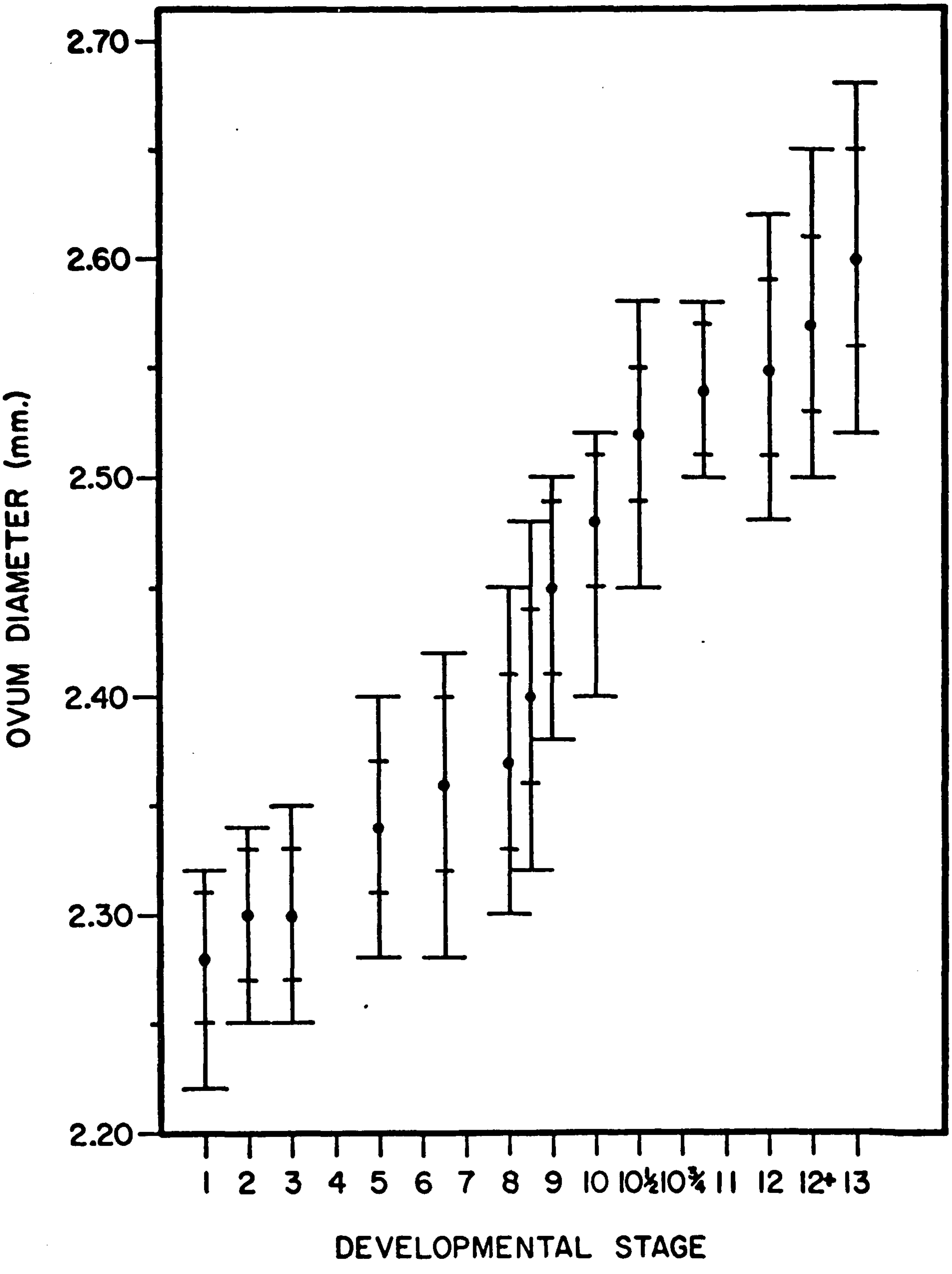
There were no intra- or interspecific differences between the increment in size up to and including the gastrula stage among the embryos of 4 *Ambystoma tigrinum* and 7 *A. maculatum* females. The size change for the embryos of 1 *A. tigrinum* female are presented in figure 1. The appropriate factors by which embryo diameters should be multiplied in order to obtain initial ovum size are presented for specific Harrison stages (Rugh, 1948) in table 1.

Development through gastrula results in a 13% increase in diameter of the embryo. In estimates of ovum volume and clutch volume the difference is even more pronounced. An error of 13% in estimating ovum diameter would result in a volume error of 43%. This large effect of ovum diameter differences on ovum volume emphasizes the need for considerable care being taken in obtaining ovum size measurements. The conversion factors reported in table 1 were applied when appropriate, and all ovum size reports that are presented (see results) take the stage of development into account.

Calorimetry

Ova and embryos were decapsulated mechanically, individually placed on glass plates and dried at 55°C in a vacuum oven for 48 h. (Reiners and Reiners, 1972). Specimens were then scraped off the glass plates with a razor blade and stored individually in glass vials in a vacuum dessicator over Drierite until subsequent analyses. Dry weights

Figure 1. Means, ranges and standard deviations (n=25) of diameters of ova and embryos at specific Harrison stages (see table 1).



OVUM DIAMETER (mm.)
2.70
2.60
2.50
2.40
2.30
2.20
1
2
3
4
5
6
7
8
9
10
10½
10¾
11
12
12+
13
DEVELOPMENTAL STAGE

Table 1. Conversion factors for finding ovum diameters from embryo diameters at specific developmental stages.

Harrison Stage	Description	Conversion Factor
1	uncleaved	1.00
2	first cleavage	0.99
3	second cleavage	0.99
4	third cleavage	0.98
5	early cleavage	0.97
6	mid- cleavage	0.97
7	late cleavage	0.97
8	blastula	0.96
9	blastula	0.93
10	dorsal lip	0.92
10½	1/3 blastopore	0.90
10¾	1/2 blastopore	0.90
11	3/4 blastopore	0.90
12	complete blastopore	0.89

were determined by weighing individual eggs and embryos to the nearest 0.01 mg. using a Mettler H20 analytical balance. The dry weight per unit volume of fresh ova (material density) was determined by dividing the average dry egg weight by the average ovum volume for the specific female.

Ash content was determined by pooling a sufficient number of eggs and combusting the dried material in a muffle furnace at 550°C for four hours (Paine, 1971; Reiners and Reiners, 1972).

Energy content of materials was determined by using a Phillipson Micro-bomb calorimeter (Phillipson, 1964) and standard calorimetric technique. Corrections were applied for fuse wire "glow" but not for acid production since the materials to be compared are so similar and the correction values would be slight (Paine, 1971). In order to minimize the temporal variability in calibration values, calorimeters (two were run simultaneously) were calibrated at the beginning and end of each day. In this way the coefficient of variability of calibration was kept to 0.01 or less. Precision of measurement is of critical importance in this type of analysis since total weights of samples are so small. Out of 33 groups of ova (average of 9 determinations per group) the average coefficient of variability is 0.026 (SD = 0.0088). Allowing for greater variability in biological materials, this amount of variability has been accepted by several authors (Golley, 1961; Paine, 1971; Calow and Jennings, 1974), and indeed significant intergroup variability in this study was not obscured (see results).

Because of the small weights of the samples and the nature of the yolky material it was necessary to combine the individual ovum or embryo with a known quantity of benzoic acid (Paine, 1971). A small quantity of benzoic acid was added to a pellet press. A previously weighed sample was then put in followed by another small amount of benzoic acid. The resulting "sandwich" was then weighed and the quantity of benzoic acid was determined by subtraction of the sample weight from the total pellet weight. As an indication of the relative amounts of sample and benzoic acid used in each pellet, 60 determinations of energy content of A. tigrinum eggs were averaged. The average ova weighed 3.8 mgs. (SD = 0.62) to which was added an average of 4.4 mgs. (SD = 0.82) of benzoic acid resulting in an average pellet weight of 8.2 mgs. (SD = 0.91). Complete combustion and low amounts of variability obtained attest to the suitability of the technique (K.C. Cummins, pers. comm.). A minimum of 6 determinations from each group of ova or embryos was built into the design of the study and achieved in almost all cases. In compiling the data it was noticed that there is a tendency for larger pellets to yield lower energy values ($r = -0.333$, $n = 60$, $p \leq 0.01$). This bias, however, is presumed to have been distributed randomly over the study so its effects should be minimized.

Dry weight-specific energy content was determined by dividing the total energy produced by the biological sample by the weight of the sample. Energy density of the ova was determined by dividing the total energy per ovum by the volume of the ovum.

Developmental rate (temperature considerations, staging and larval size)

After the ova of specific females were measured (for diameter and energy content) the remainder of the clutch, whose embryos were not yet beyond the gastrula stage, was divided into three equal parts. In the cases of A. tigrinum and A. maculatum approximately 25 embryos (with capsules intact) were placed in a finger bowl with approximately 250 ml. of 10% Holtfreter's solution (Hamburger, 1942). The pH remained near

7.0 and the solution was changed at least every three days. In the case of *A. opacum* a similar number of eggs was placed on a moist earth surface in a 250 ml. finger bowl and covered with a moist piece of filter paper. The embryos were rolled every other day in order to prevent the growth of mold. In all cases dead embryos were removed as soon as they were observed.

Each of the three groups of embryos from each female was placed in controlled temperature boxes kept at 10°C (±0.5°C), 20°C (±0.5°C) and one box which cycled between 10° and 20°C (±0.5°C) on a 12 h. cycle. Each box was kept on a 12 h. photoperiod. The 10°C temperature comes the closest to the mean natural temperature at which embryos of all populations develop. The 20°C temperature, while being somewhat high, can still be encountered in the field by all three species. In addition, the 20°C temperature has been used as a standard in other studies that deal with the embryonic development of these animals (e.g. Dempster, 1933; Moore, 1939; Hopkins and Handford, 1943; Dushane and Hutchinson, 1944). Temperatures fluctuate in nature and variations of 3° to 6°C in the aquatic habitats were not uncommon. (Temperature was continuously monitored in the case of *A. maculatum*.) Since there is no water to act as a temperature buffer for *A. opacum* clutches, greater variation is expected. This was borne out by Graham (1971) who found temperature at covered nest sites to vary on a daily basis by as much as 10°C. An alternating temperature regime (Messenger and Flitters, 1959) between 10°C and 20°C on a 12 h. cycle was chosen because of the approximation to normal temperature ranges and because the 10°C difference should be large enought to determine any effects of a variable temperature regime on various developmental parameters. The temperature cycle used is presented in figure 2. It must be kept in mind that the cycle is artificial and involves changes that are more sudden and extreme than would normally be encountered in nature.

Embryos were examined under a dissecting microscope to determine the stage of development. Such determinations were done frequently as embryos approached the desired stages (gastrula, hatching and feeding). The embryos were considered to have reached a specific stage when 50% attained the appropriate morphological condition (see Zweifel, 1968). All development times are reported from the gastrula stage. In order to standardize developmental stages among the three species, morphological as well as ecological criteria were applied. Gastrula (Harrison stage 12; Rugh, 1942) is characterized as an embryo with a completely formed blastopore. The hatching and feeding stages are more variable and in studies of non-related species may impose complications (see comment, Brown, 1977). A. maculatum always hatched at Harrison stage 40 (figure 3). (Harrison stages are based on A. maculatum embryos.) A. tigrinum, while lacking balancers and with very small anterior limb buds, hatched repeatedly with a rounded dorsal fin, many fimbrae on the external gills and a well defined ventral abdominal vein, all of which are characteristic of Harrison stage 40. However, it has long been known (see Noble and Brady, 1933) that A. opacum can hatch over a range of developmental stages depending on the time of inundation of the clutch. In order to allow for meaningful interspecific comparisons, A. opacum embryos were immersed in 10% Holtfreter's solution at approximately stages 38-39, which allowed for their hatching at stage 40 which closely resembles A. maculatum morphologically.

Attainment of the feeding stage was determined when the larvae

Figure 2. Pattern of alternating temperature used in developmental studies.

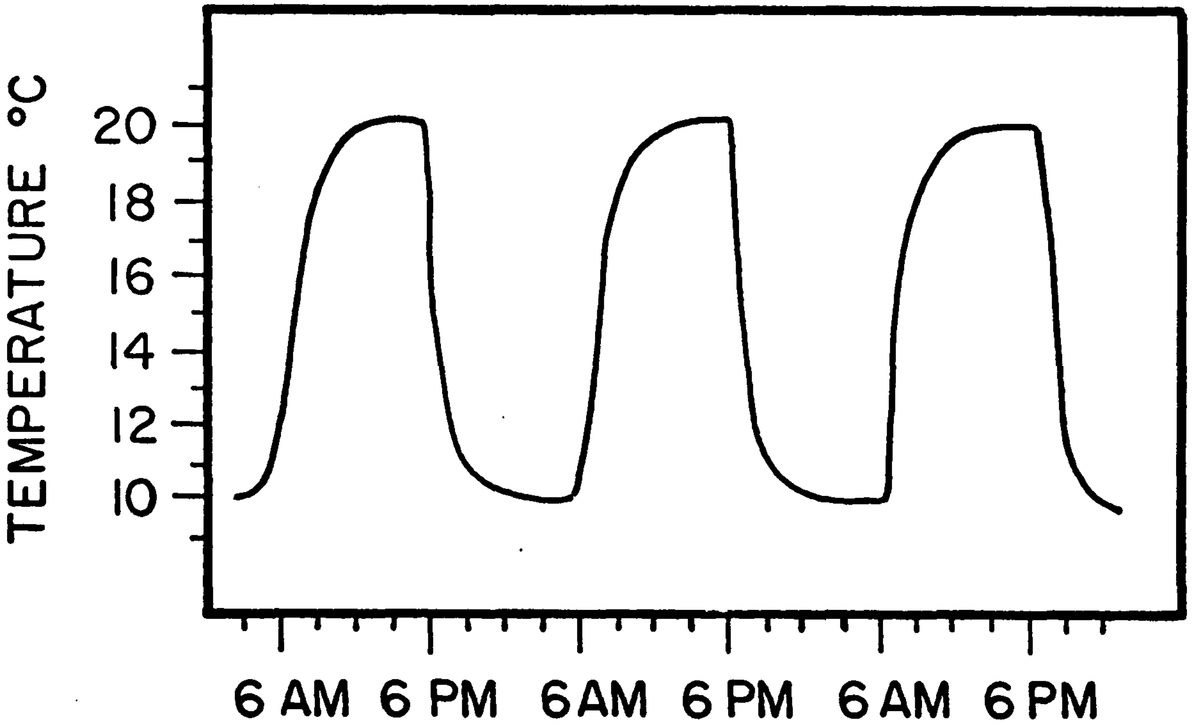
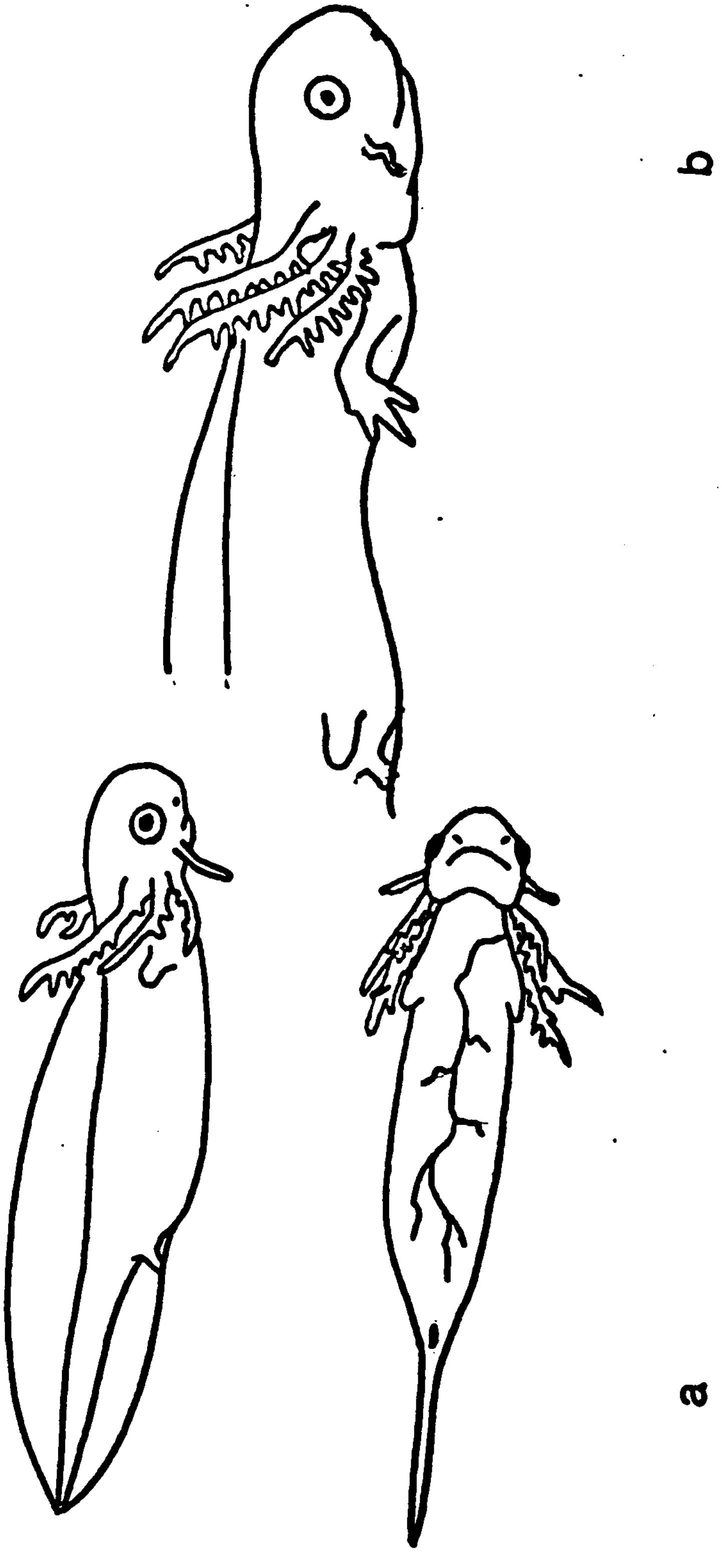

TEMPERATURE °C
20
18
16
14
12
10
6 AM
6 PM
6 AM
6 PM
6 AM
6 PM

Figure 3a. Hatching (Harrison stage 40) stage of *Ambystoma maculatum*.

b. Feeding (Harrison stage 46). (After Rugh, 1942).


b
a

snapped at and retained food (tubifew worms). This takes place at stage 46 (figure 3) in A. maculatum and A. opacum, both being nearly identical, with A. maculatum having a slightly more pronounced hind limb bud. Balancers are shrivelled or gone and the fourth digit on the forelimb is barely visible (as reported also by Stultz, 1936). The forelimb of the A. tigrinum feedling is similar to that of A. maculatum at stage 40 (figure 3). Hindlimbs are absent. All three species have used almost all their yolk by the time feeding begins and the gut takes on a thin, convoluted appearance. Despite the differences between species, the degree of yolk utilization and general morphology (gills, circulation, and musculature) make the utilization of the chosen stages appropriate. Of course the hatching and feeding stages are both ecologically important.

As 50% of the embryos and larvae of each female at each temperature attained the hatching and feeding stages, 10 larvae were randomly selected and total length determined by using a dissecting scope and a millimeter rule. The larvae were removed (without harm) from their aquatic environment to obtain these measurements. In this way approximately 5400 larvae were measured during the course of this study. In addition, at these stages, six larvae of each female at each temperature were sampled randomly and dried, weighed, and analyzed calorimetrically, as described above.

Respirometry

Respiratory rates were determined at the hatching and feeding stages for both A. maculatum and A. tigrinum larvae that developed under constant temperature conditions (10°and 20°C). (Lack of sufficient individuals prevented rates from being established for

A. opacum.) Three larvae were sampled randomly from the group as described above. The larvae were then tested individually and mean oxygen consumption rates (ul O_2/h.) were determined.

Respiratory rates were determined with a Rank Oxygen Electrode of the type used by Calow (1975). The electrode was operated at a 0.6-V polarization potential. The reaction chamber is cylindrical with a depth of 40 mm., a diameter of 16 mm., and a maximum capacity of 5.0 ml. The reaction chamber was filled with 2 ml. of 10% Holtfreter's solution, saturated with air at the test temperature by bubbling air through it for at least 24 hours prior to use. The chamber was maintained at a constant temperature by a water jacket housing. In order to allow for complete diffusion of oxygen a Tygon collar, which fit snugly into the chamber, supported a plastic mesh which prevented the larvae from contacting an automatic stirrer. Oxygen diffused through a thin Teflon membrance and a Pt/O_2 half cell was joined to a Ag/AgCl half cell by an electrolyte bridge consisting of lens tissue soaked in 50% saturated KCl. Under conditions of constant diffusion rate the electrode current is proportional to the oxygen concentration in the solution, i.e. the potentiometric deflection is linear between 0 - 100% oxygen concentration (Calow, 1975). After the Teflon membrane was changed (ca. every two to three weeks) the electrode was allowed to stabilize for 5 to 10 hours. Each day before operation it was stabilized for 1½ hours.

Larvae were placed in the reaction chamber and the top sealed. Each run lasted 20 to 30 minutes but in all cases only the last 15 minutes were used in calculation of rate in order to avoid the effects of handling. After approximately 10 minutes in the chamber the O_2

consumption rate was linear for up to an hour and was not affected by oxygen concentration until the concentration dropped to 30% of that of air-saturated water. Potentiometric deflection per unit time was converted to ul/O_2 per hour based on the assumption that the 10% Holtfreter's solution was air-saturated at the test temperature and that barometric pressure did not differ appreciably from 1 atmosphere. Since test larvae were allowed freedom to move but were quiescent during measurement, and since they were all at the same nutritional state, the rates reported represent standard metabolic rates (Dejours, 1975).

Post-feeding growth

After the feeding stage was reached, the remaining larvae were separated into smaller groups (4 feedlings per group) and kept with an unlimited supply of tubifex worms (which they readily consumed) in 250 ml. finger bowls with 10% Holtfreter's solution. The solution was changed at least every three days. Ten growing larvae were sampled randomly and length measurements were made at weekly intervals. In this way post-feeding growth was followed for a three week period.

Statistics

In the analysis of body volume, ovum volume, clutch size and clutch volume presented below, the data were log-transformed to achieve linearity and were then checked for normality (nonsignificant skew or kurtosis at the 5% level) before applying statistical tests. Straight lines were fitted to the data by the technique of least squares regression. This proves adequate where correlations are high (Gould, 1975a).

In subsequent analyses, parametric analyses of variance were only applied when the data were shown to be homoscedastic ($p \leq 0.05$).

Otherwise non-parametric analyses of variance (Kruskal-Wallis test; Sokal and Rohlf, 1969) were applied. Before product-moment correlations and t-values were calculated that data were also checked for normality.

RESULTS

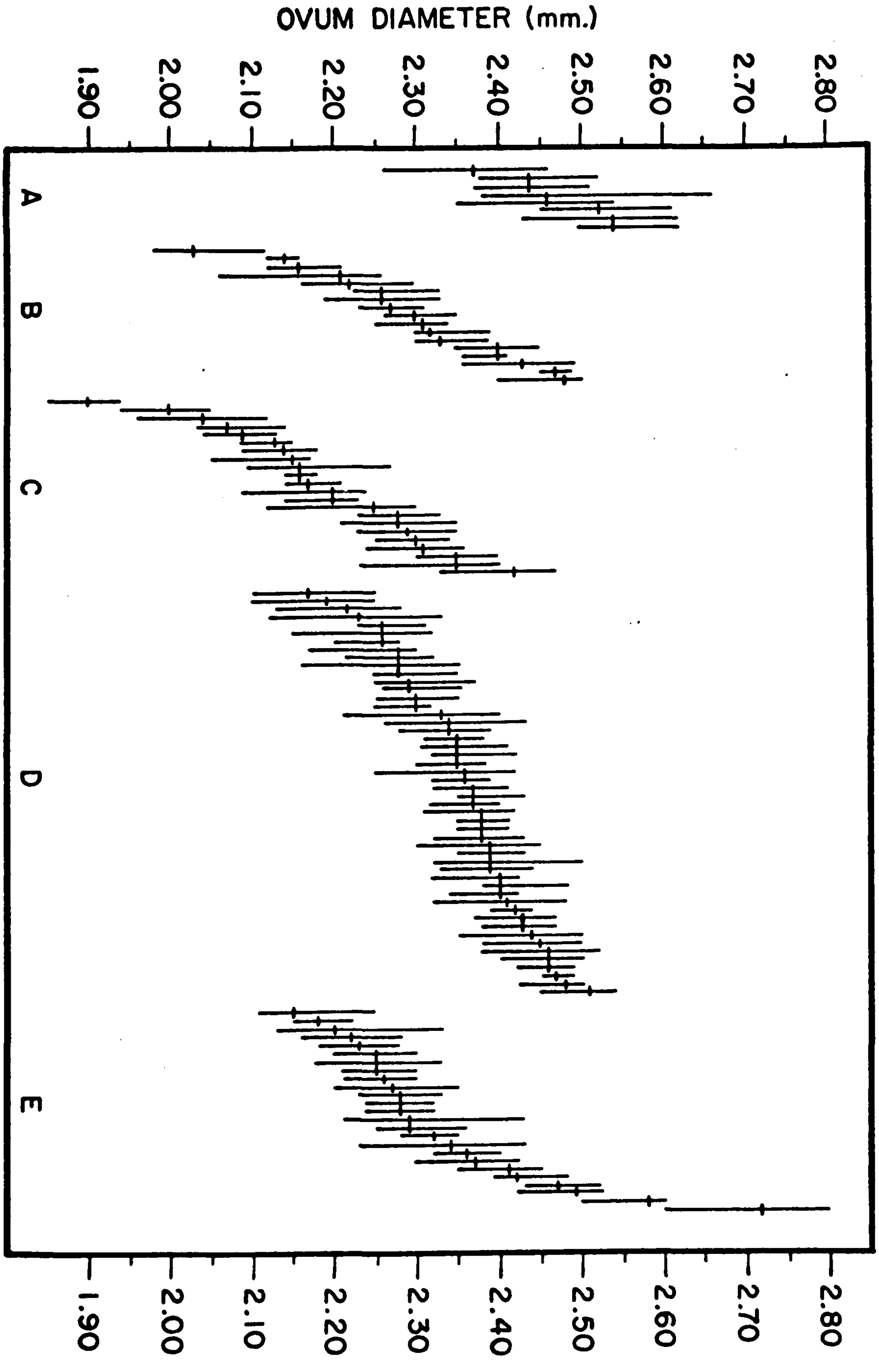
Variability in ovum size among females within a population is the focus of this study. The traditional measure of egg size (ovum diameter) is useful in that it allows for comparisons between individuals and populations. From a functional point of view, however, ovum diameters per se do not convey much information either about the relative space an egg or egg mass occupies or about the relative differences in the amount of material and energy that is available for the construction of a larva. Data on ovum diameters will be presented at the outset. Ovum volumes will then be considered and related, first to characteristics that are most germane to the female parent (e.g., adult body size, clutch size, and clutch volume), and then to characteristics that are most relevant from the point of view of the offspring (e.g., developmental rate, size and stage of larvae at hatching and feeding, the energetic cost of development, the efficiency of development, respiratory rate and post-feeding growth). In this way egg size links the reproductive strategy of the parent to the developmental strategy of the resulting offspring.

OVUM SIZE

Intra- and interpopulational variability

Adjusted mean ovum diameters and ranges (see materials and methods) for the five populations of Ambystoma studied are arranged in ascending order in figure 4. Non-parametric analysis of variance (Kruskal-Wallis test) indicates that within each of the five populations there are significant differences between ovum sizes among the individuals ($p \leq 0.005$, in all cases). If individual values are pooled and popula-

Figure 4. Means and ranges for ovum diameters of individual females arranged in ascending order. A = *A. opacum*$_{Ga.}$; B = *A. opacum* S.C.; C = *A. opacum*$_{N.J.}$; D = *A. maculatum*; E = *A. tigrinum*.


OVUM DIAMETER (mm.)
1.90
2.00
2.10
2.20
2.30
2.40
2.50
2.60
2.70
2.80
A
B
C
D
E

tion differences considered non-parametrically (figure 5), significant differences between populations are found ($\chi^2 = 37.6$, $p \leq 0.005$). This is despite there being less than a 0.30 mm. difference in diameter between the population with the largest eggs (*A. opacum*-Ga., X = 2.47 mm., SD = 0.059) and that with the smallest (*A. opacum*-N.J., X = 2.19 mm., SD = 0.127).

If ovum volume is considered, rather than ovum diameter, the significance of the differences among individuals and among populations (figure 6) is maintained, but made more obvious. A 0.05 mm. difference in ovum diameter, which is found among the individuals in the *A. opacum*-N.J. population for example, results in functional egg size (e.g. volume) being doubled between the females that produce the largest and those that produce the smallest ova. Functional egg size, therefore, must be considered when making comparisons of the interaction of egg size either with other female parameters or with offspring parameters.

THE FEMALE

Body size, clutch size, and clutch volume variability (intra- and interpopulational)

It is clear from figure 7 that the four populations for which there are appropriate data differ in the amount of space they contain within their bodies ($\chi^2 = 84.6$, p 0.005). Presumably these body differences will relate to clutch parameters (clutch size, ovum size, and clutch volume), some at least of which are expected to be larger in individuals from populations with larger mean body sizes. Female-specific clutch sizes and ovum volumes are multiplied together to

Figure 5. Means, ranges and 95% confidence limits of mean ovum diameters for 5 populations of *Ambystoma*. A = *A. opacum*$_{\mathrm{Ga.}}$; B = *A. opacum*$_{\mathrm{S.C.}}$; C = *A. opacum*$_{\mathrm{N.J.}}$; D = *A. maculatum*; E = *A. tigrinum*.

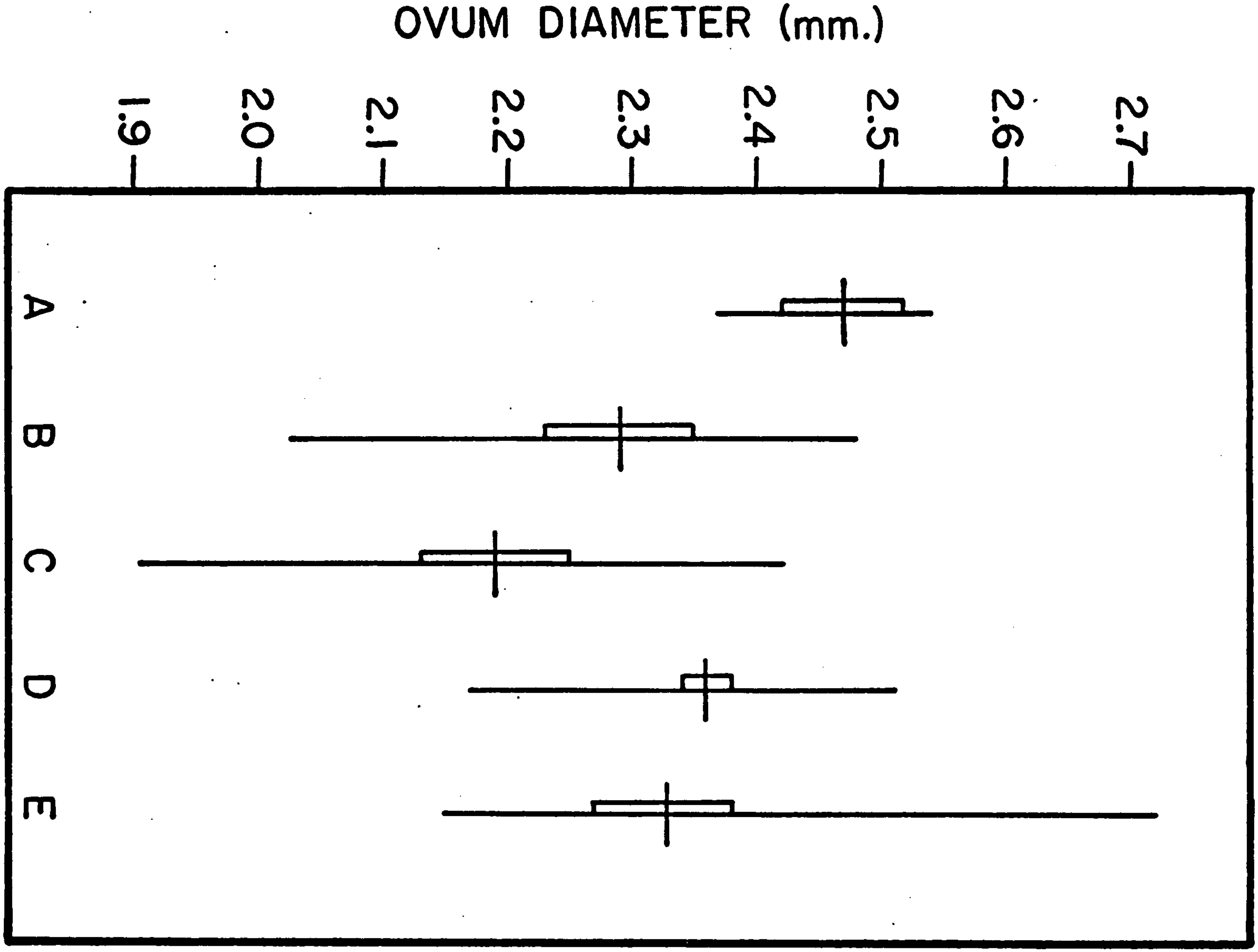

OVUM DIAMETER (mm.)
1.9
2.0
2.1
2.2
2.3
2.4
2.5
2.6
2.7
A
B
C
D
E

Figure 6. Means, ranges and 95% confidence limits of mean ovum volumes for 5 populations of _Ambystoma_. (See figure 5)

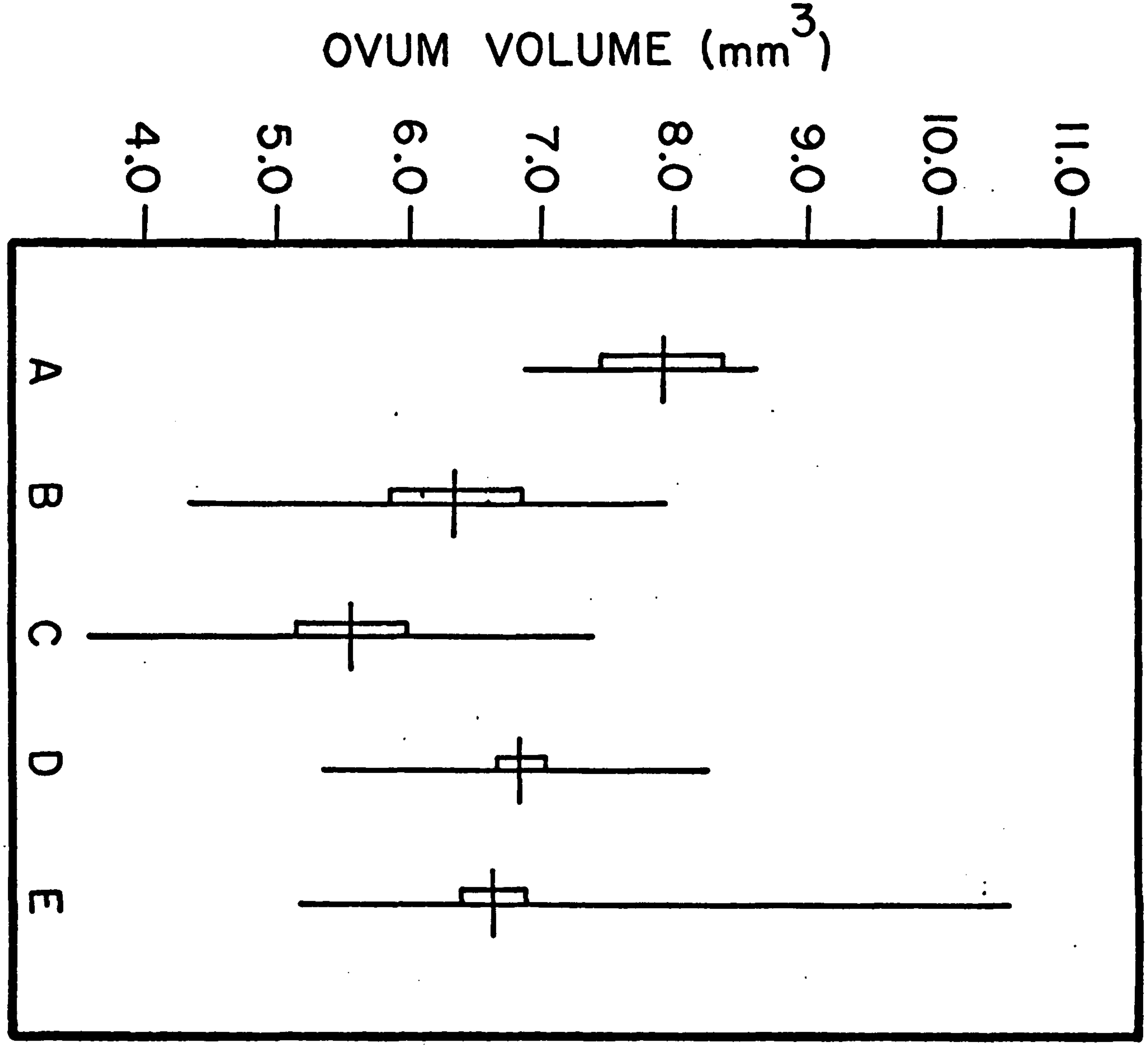
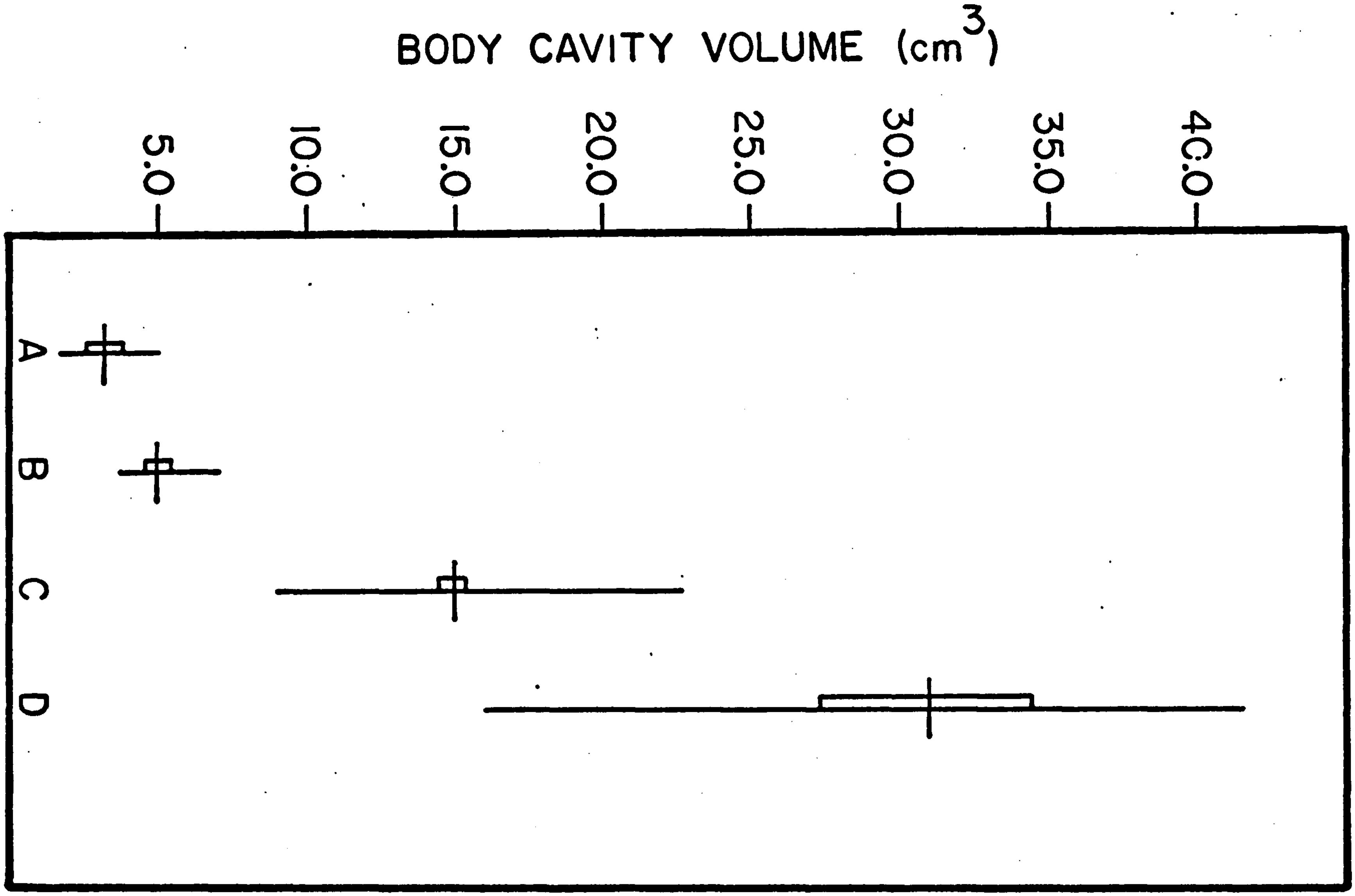

OVUM VOLUME (mm^3)
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
A
B
C
D
E

Figure 7. Means, ranges and 95% confidence limits of mean body cavity volumes for 4 populations of *Ambystoma*.

A = *A. opacum*$_{S.C.}$; B = *A. opacum*$_{N.J.}$; C = *A. maculatum*; D = *A. tigrinum*.


BODY CAVITY VOLUME (cm^3)
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
A
B
C
D

obtain female-specific clutch volumes. Ovum size is very conservative in these four populations of Ambystoma and the significant differences that do occur in ovum volume (figure 6) are not adequately explained by body size differences. Nevertheless, clutch size (figure 8) and total clutch volume (figure 9) differences that occur among these four populations do tend to follow body size trends. The important question that needs to be answered, however, is whether the differences in clutch parameters that exist can be explained by body size differences alone or are other, perhaps ecologically important, factors involved? In order to answer this question it is necessary to establish first how clutch parameters are expected to change with body size, given the design constraints of the animals.

Body size and clutch parameter variability (interspecific)

The interspecific data presented in this section are those of Salthe (1969) with consideration of some additional recent material (Tilley, 1968; Bruce, 1972 and 1975; Peacock and Nussbaum, 1973; Bell and Lawton, 1975; Houck, 1977a). Literature reports of egg counts and ovum diameters are considered in order to determine a mean value characteristic for each species. Although data of this sort are heterogeneous with respect to number of observations and the precision of the measurements, I am confident that the regression equation of total clutch volume (calculated for mean clutch size and ovum diameter) on body volume for 74 species of salamanders (see figure 10 and below) provides a reasonable estimate for the group and will prove to be robust to new additions (as it has already) for two reasons. First, the large number of data points tends to diminish the effect of a few

Figure 8. Means, ranges and 95% confidence limits of mean clutch sizes for 4 populations of *Ambystoma*. (See figure 7.)

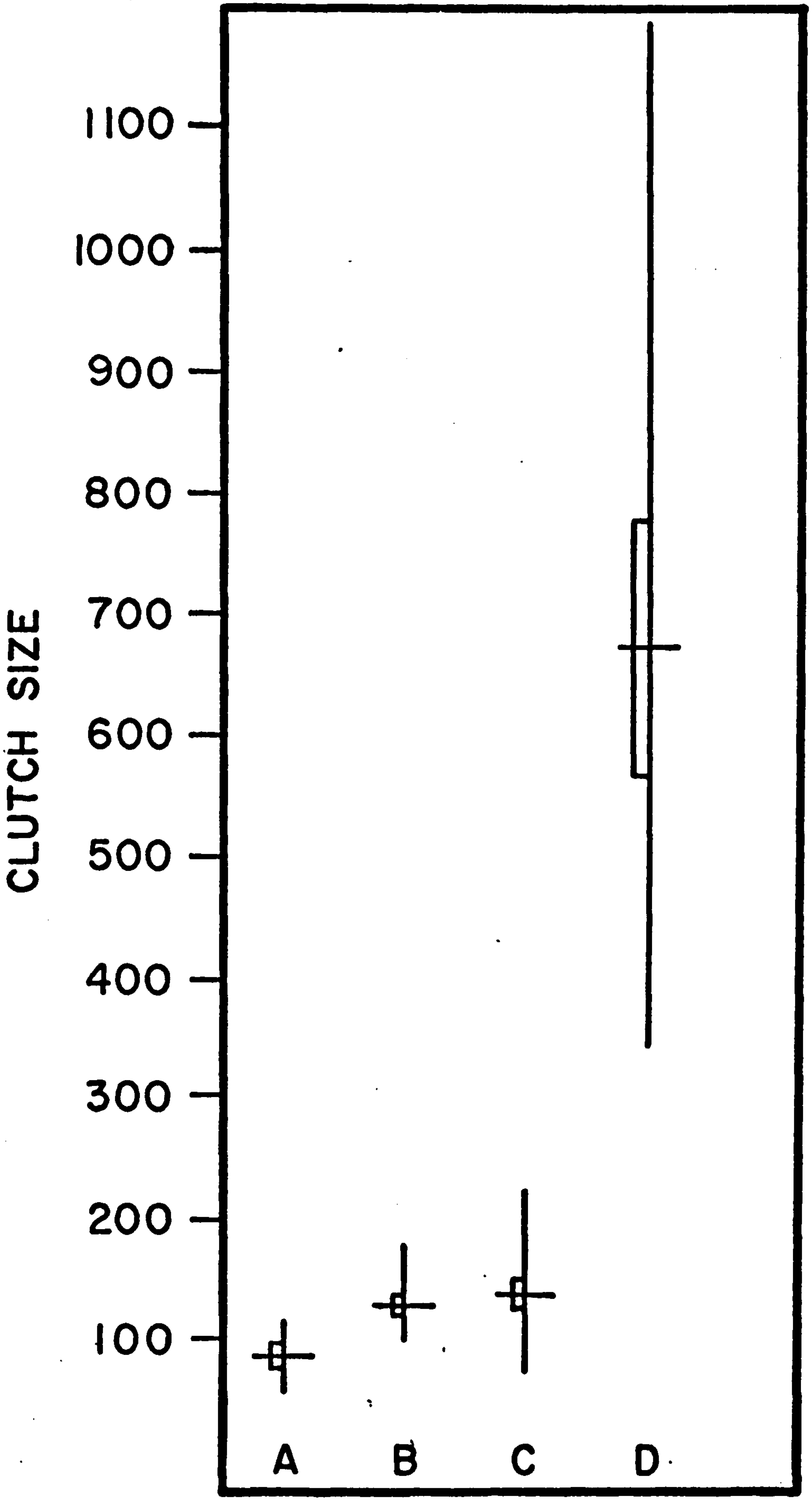

CLUTCH SIZE
1100
1000
900
800
700
600
500
400
300
200
100
A
B
C
D

Figure 9. Means, ranges and 95% confidence limits of mean clutch volumes for 4 populations of *Ambystoma*. (See figure 7.)

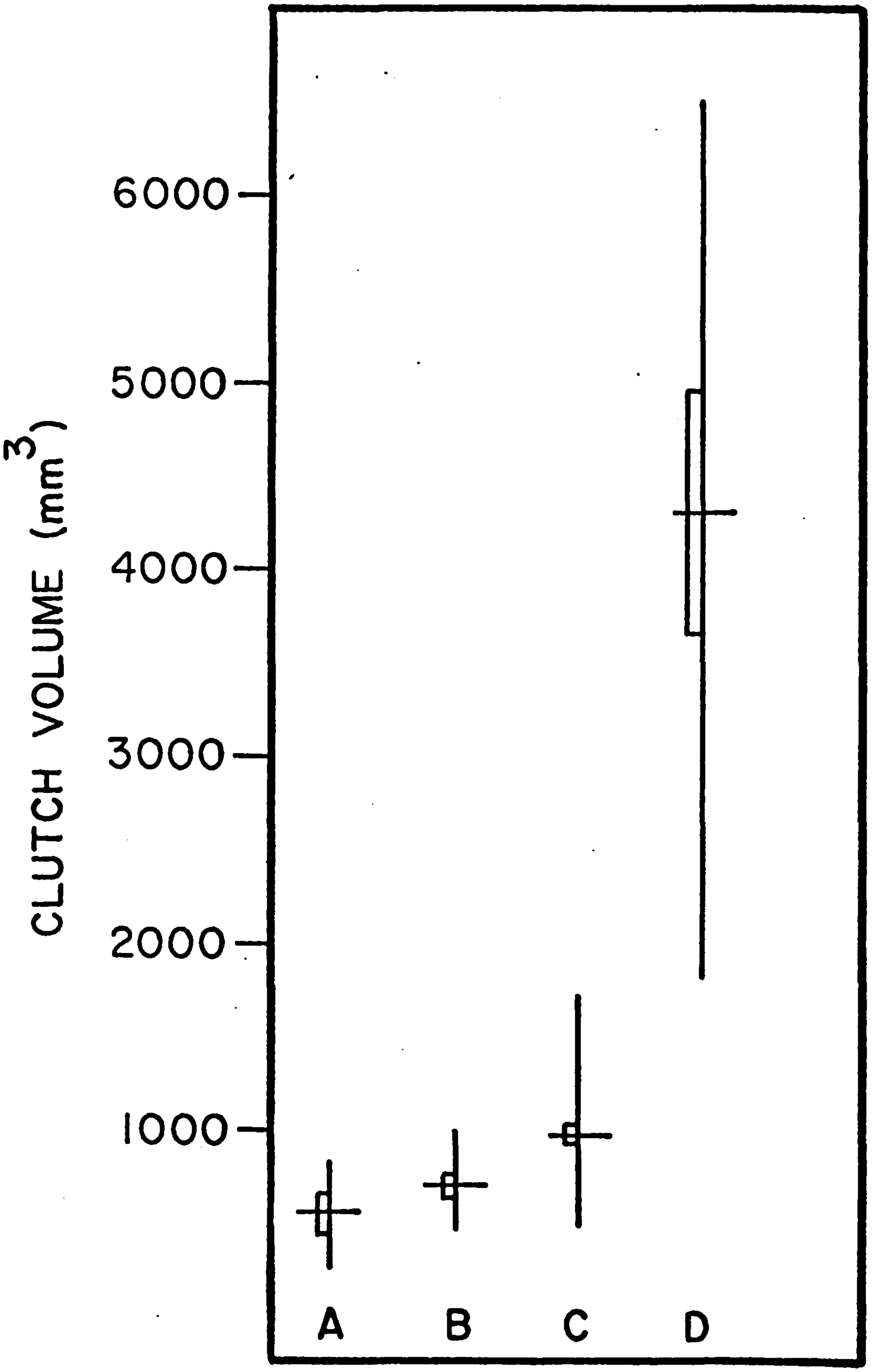
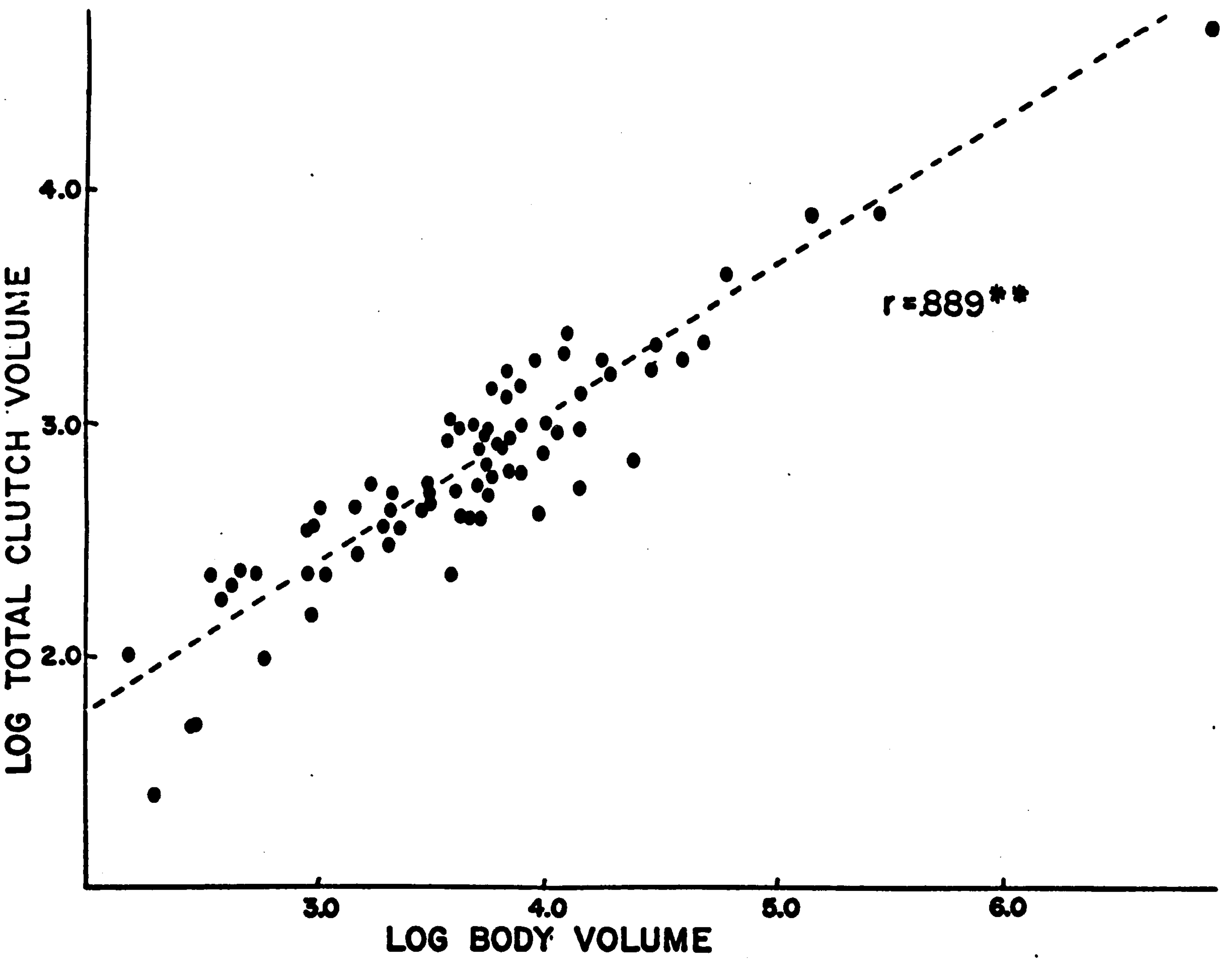

CLUTCH VOLUME (mm^3)
6000
5000
4000
3000
2000
1000
A
B
C
D

Figure 10. Log mean clutch volume (mm^3) plotted against log mean body volume (mm^3) for 74 salamander species. (** = $p \leq 0.01$).

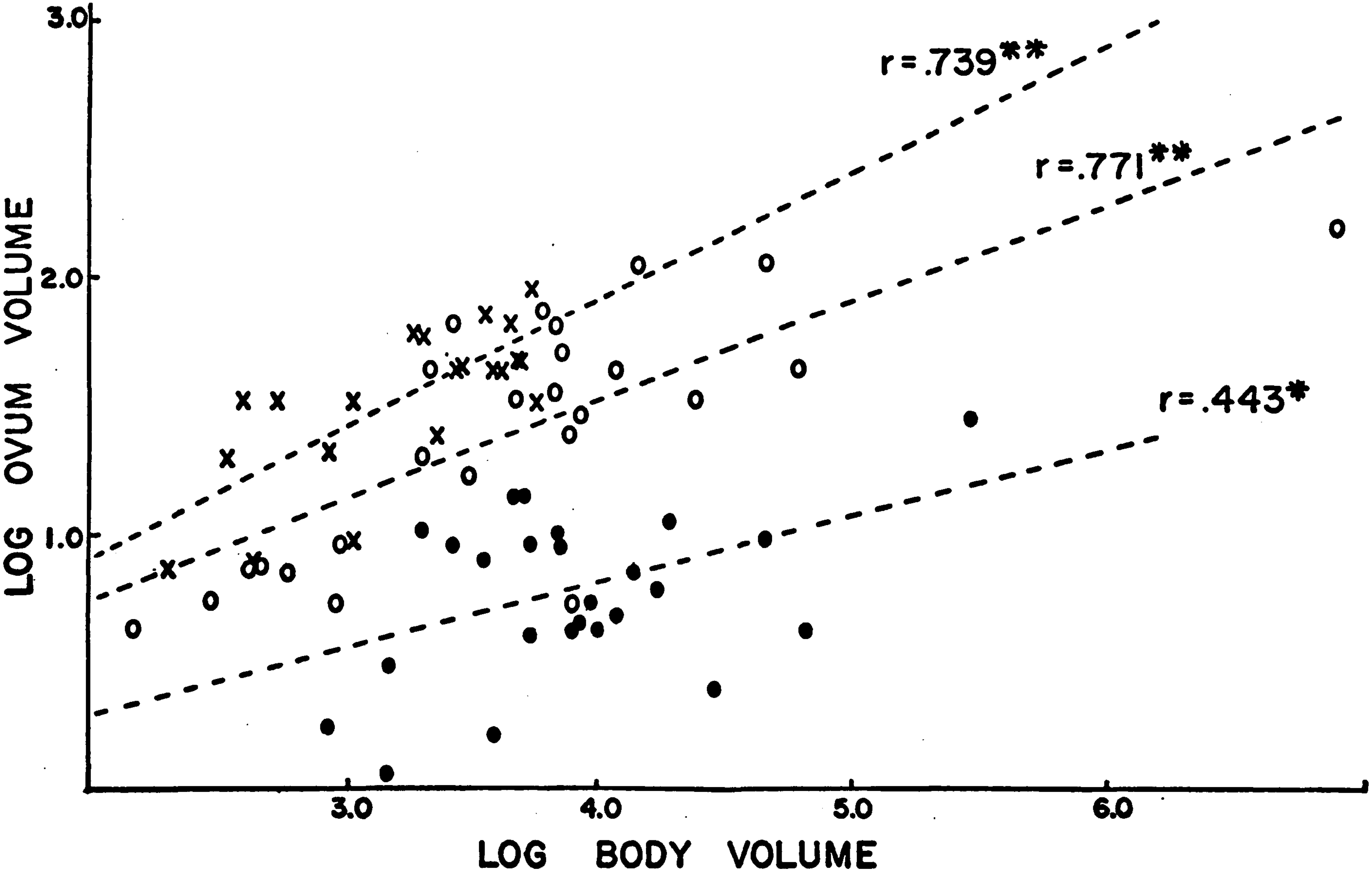

LOG TOTAL CLUTCH VOLUME
4.0
3.0
2.0
r=.889**
3.0
4.0
5.0
6.0
LOG BODY VOLUME

inaccurate values, if any exist. Second, there is a strong positive correlation in this relationship ($r = 0.899$) which would be unexpected if the data from which the relationship is constructed are not well founded.

Most salamander species can be assigned to one of three major reproductive modes (Salthe, 1969). Species included in what may be the most primitive mode (mode I) deposit their eggs in lentic waters. They are attached to submerged supports (often vegetation) either singly or in clusters. Mode II is shared by species breeding in or near running water. The female deposits eggs in hidden nests which in some species are attended by a parent. In mode III the typical aquatic larval stage is bypassed entirely and the embryos develop directly into the adult terrestrial form in nests on land, usually attended by the female.

Figure 11 shows that the different reproductive modes have characteristic ovum sizes. For any given body size, pond breeders have the smallest eggs and species showing direct development the largest. Within each mode both ovum size and egg number (not shown) tend to increase with increase in body size. Not surprisingly, clutch size tends to be reciprocally related to ovum size. For example, mode I salamanders, with small eggs, produce many of them and mode III species with large eggs produce fewer. Figure 10 shows the relationship between total clutch volume and body volume for 74 salamander species. There is no modal variability in this relationship. The high correlation suggests that body size is a significant constraint on total reproductive output at any given time regardless of ecological differences between modes. The variability around the line indicates that

Figure 11. Log mean ovum volume (mm^3) plotted against log mean body volume (mm^3) for 74 salamander species of three reproductive modes. Closed circles = pond breeders. Open circles = stream breeders. Crosses = direct developers. (* = $p \leq 0.05$; ** = $p \leq 0.01$).


3.0
2.0
1.0
LOG OVUM VOLUME
r = .739**
r = .771**
r = .443*
3.0
4.0
5.0
6.0
LOG BODY VOLUME

within a narrow range of body sizes some species approach a potentially maximal output to a greater extent than do others. The slope of this relationship (a = 0.640) shows that as body size increases, size specific reproductive output decreases. This latter point has been overlooked (due to insufficient interspecific data) in many analyses of reproductive effort differences between closely related species where similar measurements were utilized (among salamander studies, see for example, Tilley, 1968 and Wilbur, 1977).

If there is a body size constraint on reproductive output, a significant trade-off between the components of reproductive output would be expected. Ovum size cannot be increased without incurring a cost in decreased egg number unless body size also changes. This is indicated by a partial correlation coefficient of -0.852 between ovum size and egg number for salamanders of a given body size. More than 70% of the variability in the one parameter is explained by variability in the other. This relationship is also reflected in figures 12 and 13 which show clutch size and ovum volume plotted against body volume for mode I salamanders alone. Note that hynobiids, with small clutch sizes, have correspondingly large ovum sizes while salamandrids, on the other hand, have very small ova and relatively many of them. Hence, within a reproductive mode there are taxonomic comparisons of interest.

The interspecific data thus far presented indicate that a salamander of a given body size can have a predictable portion of its body volume occupied by ova. Its clutch will be divided into eggs of a size strongly dependent upon its particular mode of reproduction. Within a particular reproductive mode, clutch size and ovum volume are further determined to some extent by phylogenetic constraints, presum-

Figure 12. Log mean clutch size plotted against log mean body volume (mm^3) for mode I (pond breeding) species. Closed circles = Ambystomatidae. H = Hynobiidae. N = Salamandridae. S = Sirenidae. ($* = p \leq 0.05$).

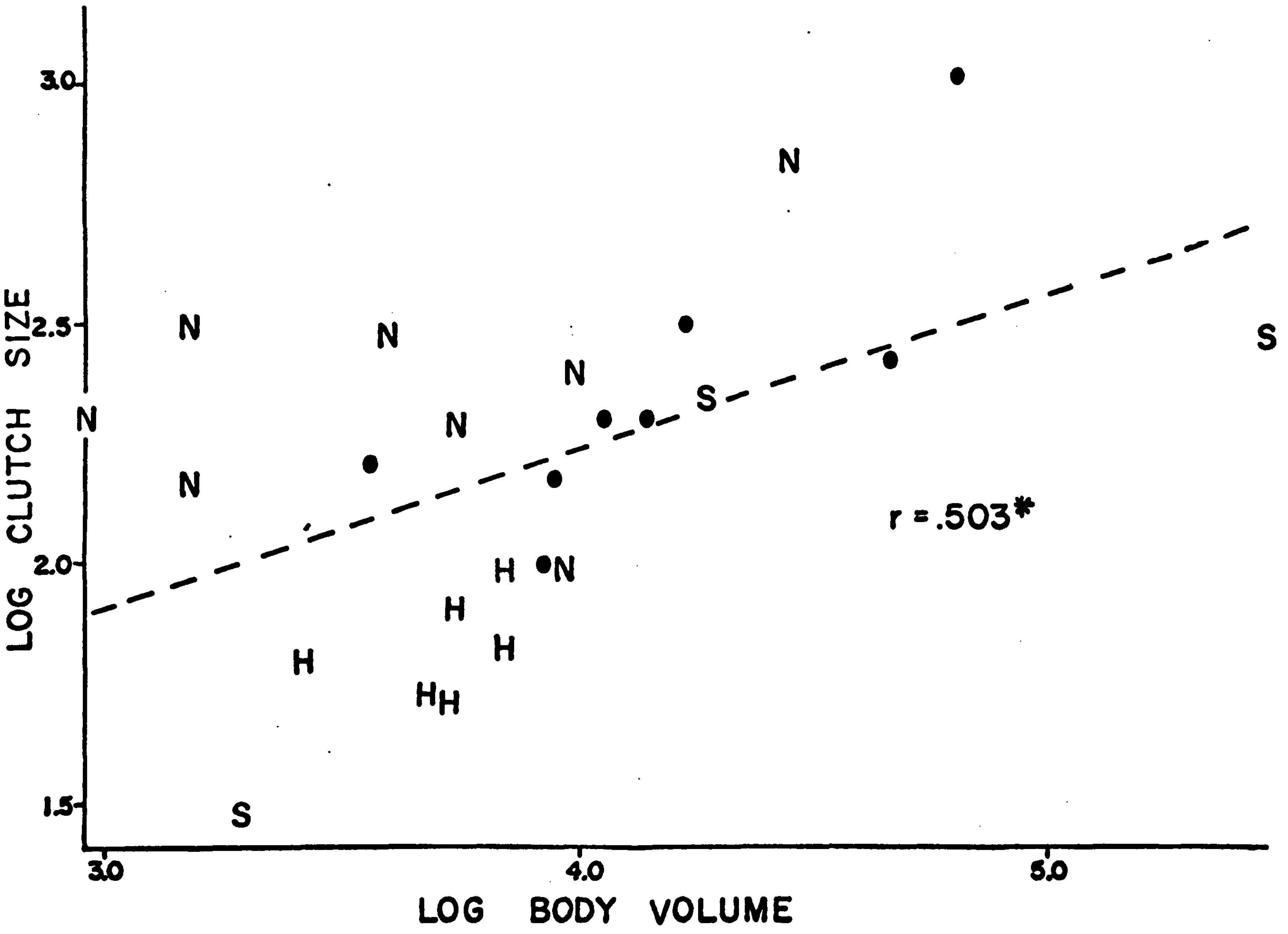
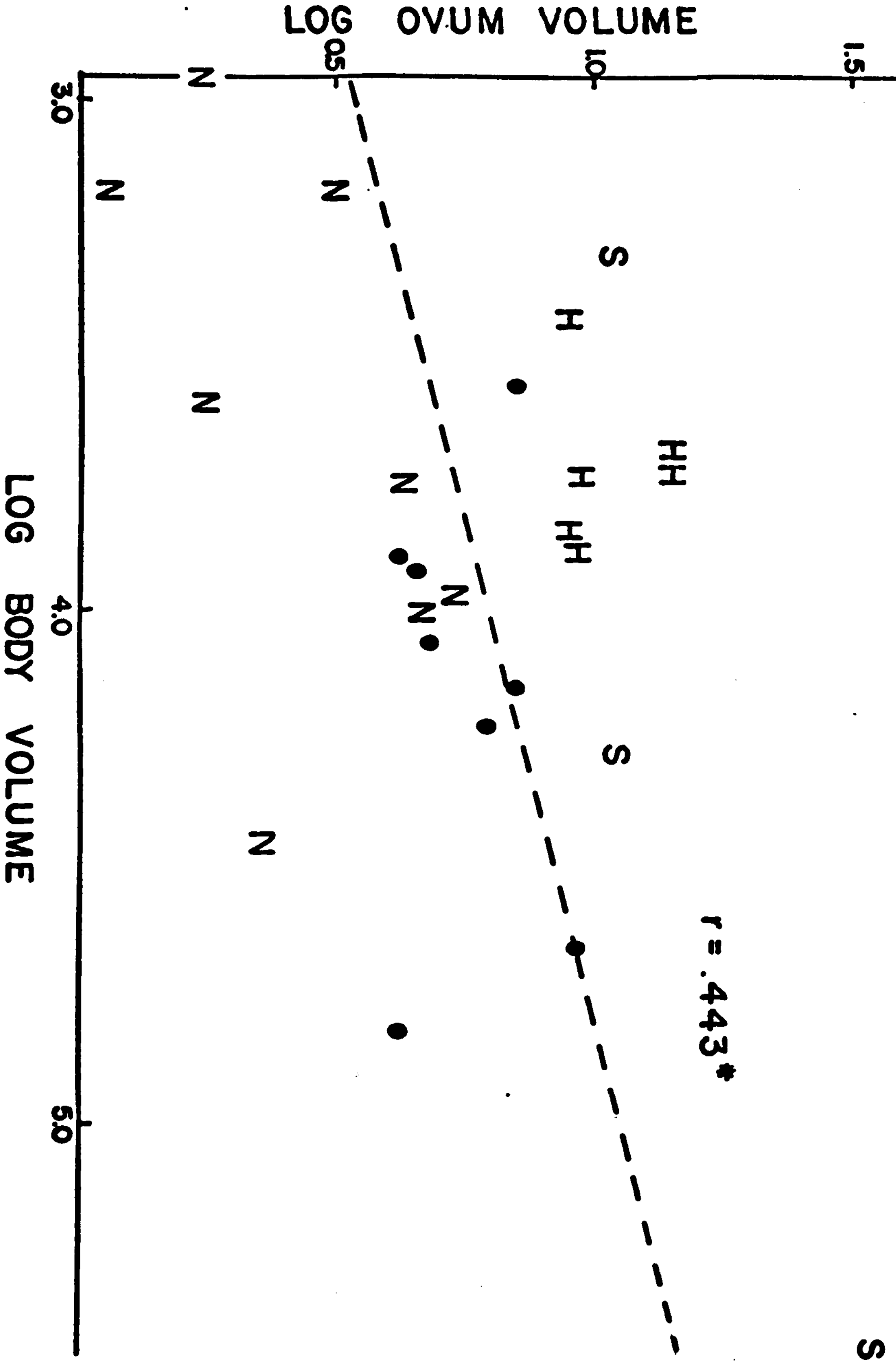

LOG CLUTCH SIZE
3.0
2.5
2.0
1.5
3.0
4.0
5.0
LOG BODY VOLUME
N
H
S
r = .503*

Figure 13. Log mean ovum volume (mm^3) plotted against log mean body volume (mm^3) for mode I (pond breeding) species. (Symbols as in figure 12).


LOG OVUM VOLUME
0.5
1.0
1.5
LOG BODY VOLUME
3.0
4.0
5.0
N
H
HH
S
r = .443*

ably reflecting differences in taxon-related reproductive adaptations.

Interspecific models and populational comparisons

Models can be constructed which relate body size to a particular reproductive character. The clutch volume - body volume relationship observed in each of the four populations studied is plotted in figure 14, which also indicates the relationship found among all salamander species (from figure 10). This figure indicates graphically that A. tigrinum produces a larger than predicted clutch volume and that A. maculatum has a smaller one than expected on the basis of the body size model. Neither A. opacum population is unusual in this regard.

The deviation of the mean log clutch volume (for a salamander having the mean log body size in the test population) from the log clutch volume predicted for one of the same size from the model was tested using a t-test (see table 2). Where the deviation is significant a deviation quotient (D.Q. = the ratio of actual clutch parameter to expected clutch parameter value) can be used to describe that population's deviation (tables 2 through 4). This is directly analogous to Jerison's (1973) encephalization quotient. If the deviation is nonsignificant the graphical relationship of the population with the model can still be used to show its direction (figure 14 through 16).

Even though a population does not deviate from the model on the total clutch volume character it may deviate from models based on the components of clutch volume. Furthermore, a deviation from the clutch volume - body volume model does not indicate the source of the deviation among the components. As data accumulate several hierarchical models become possible. For example, a species model may be constructed

Table 2

Clutch volume statistics for both model and test populations.*

	n # species or individuals	$\bar{X}$ mean body volume ± S.E. (log mm^3)	$\bar{Y}$ mean clutch vol. ± S.E. (log mm^3)	(a) slope	(r) correlation	$\bar{Y}m$ at $\bar{X}p$ predicted clutch vol. for mean population body size (log mm^3)	$\bar{Y}p - \bar{Y}m$ at $\bar{X}p$ difference actual clutch vol. from predicted ± S.E. (log mm^3)	Deviation Quotient (antilog of difference)
Interspecific Model (m)	74	3.614 ± 0.116	2.779 ± 0.053	0.64	0.90 ($p < 0.01$)	———	———	———
Test Populations (p)								
A. tigrinum	15	4.487 ± 0.029	3.618 ± 0.036	1.15	0.92 ($p < 0.01$)	3.340	0.278 ± 0.034 ($p < 0.001$)	1.90
A. maculatum	48	4.164 ± 0.013	2.972 ± 0.019	0.83	0.57 ($p < 0.05$)	3.133	-0.161 ± 0.031 ($p < 0.001$)	0.69
A. opacum (N.J.)	22	3.702 ± 0.015	2.840 ± 0.018	0.55	0.47 ($p < 0.05$)	2.837	0.003 ± 0.028 (NS)	1.01
A. opacum (S.C.)	14	3.482 ± 0.040	2.709 ± 0.044	0.99	0.90 ($p < 0.01$)	2.696	0.013 ± 0.031 (NS)	1.03

* t-test: $t = \frac{\text{difference}}{\text{S.E.}} =$

$$\frac{\bar{Y}p - \bar{Y}m\,\text{at}\,\bar{X}p}{\left[\left(\frac{n_p-1}{n_p-2}\right)\left(SE^2_{\bar{Y}p}\right)\left(1-r^2_p\right)+\left(\frac{n_m-1}{n_m-2}\right)\left(SE^2_{\bar{Y}m}\right)\left(1-r^2_m\right)\left(1+\frac{(\bar{X}m-\bar{X}p)^2}{(SE^2_{\bar{X}m})(n_m-1)}\right)\right]^{1/2}}$$

with $n_m + n_p - 4$ degrees of freedom. (This equation is presented to enable recalculation of the t-values used here, and to allow use of this model with other data).

Table 3

Clutch size statistics for both model and test populations.*

	n # species or individuals	$\bar{X}$ mean body volume ± S.E. (log mm^3)	$\bar{Y}$ mean clutch size ± S.E. (log egg #)	(a) slope	(r) correlation	$\bar{Y}m$ at $\bar{X}p$ predicted clutch size for mean population body size (log egg #)	$\bar{Y}p-\bar{Y}m$ at $\bar{X}p$ difference actual clutch size from predicted ± S.E. (log egg #)	Deviation Quotient (antilog of difference)
Interspecific Model (m)	25	3.909 ± 0.111	2.207 ± 0.072	0.33	0.50 ($p<0.05$)	———	———	———
Test Populations (p)								
A. tigrinum	15	4.487 ± 0.029	2.817 ± 0.033	0.97	0.84 ($p<0.01$)	2.397	0.420 ± 0.095 ($p<0.001$)	2.63
A. maculatum	48	4.164 ± 0.013	2.124 ± 0.017	0.57	0.44 ($p<0.01$)	2.291	-0.167 ± 0.072 ($p<0.05$)	0.68
A. opacum (N.J.)	22	3.702 ± 0.015	2.101 ± 0.014	0.24	0.27 (NS)	2.141	0.040 ± 0.069 (NS)	1.10
A. opacum (S.C.)	14	3.482 ± 0.040	1.916 ± 0.028	0.61	0.88 ($p<0.01$)	2.069	-0.153 ± 0.082 ($p<0.10$)	0.70

* See explanation to Table 2 for statistical test used.

Table 4

Ovum volume statistics for both model and test populations.*

	n # species or individuals	$\bar{X}$ mean body volume ± S.E. (log mm^3)	$\bar{Y}$ mean ovum vol. ± S.E. (log mm^3)	(a) slope	(r) correlation	$\bar{Y}m$ at $\bar{X}p$ predicted ovum vol. for mean population body size (log mm^3)	$\bar{Y}p - \bar{Y}m$ at $\bar{X}p$ difference actual ovum vol. from predicted ± S.E. (log mm^3)	Deviation Quotient (antilog of difference)
Interspecific Model (m)	25	3.909 ± 0.111	0.763 ± 0.065	0.26	0.44 (p 0.05)	—	—	—
Test Populations (p)								
A. tigrinum	15	4.487 ± 0.029	0.801 ± 0.016	0.19	0.33 (NS)	0.913	-0.112 ± 0.088 (NS)	0.77
A. maculatum	48	4.164 ± 0.013	0.848 ± 0.007	0.27	0.54 ($p<0.01$)	0.829	0.019 ± 0.066 (NS)	1.04
A. opacum (N.J.)	22	3.702 ± 0.015	0.738 ± 0.017	0.31	0.29 (NS)	0.710	0.028 ± 0.066 (NS)	1.07
A. opacum (S.C.)	14	3.482 ± 0.040	0.792 ± 0.020	0.38	0.78 ($p<0.01$)	0.653	0.139 ± 0.077 ($p<0.10$)	1.38

* See explanation to Table 2 for statistical test used.

Figure 14. Log clutch volume (mm^3) plotted against log body volume (mm^3) for individuals of four populations of *Ambystoma*. Triangles = *A. opacum*$_{S.C.}$; open circles = *A. opacum*$_{N.J.}$; closed circles = *A. maculatum*; crosses = *A. tigrinum*. Dashed line is the model and is taken from figure 10. * = p 0.05; ** = p 0.01.

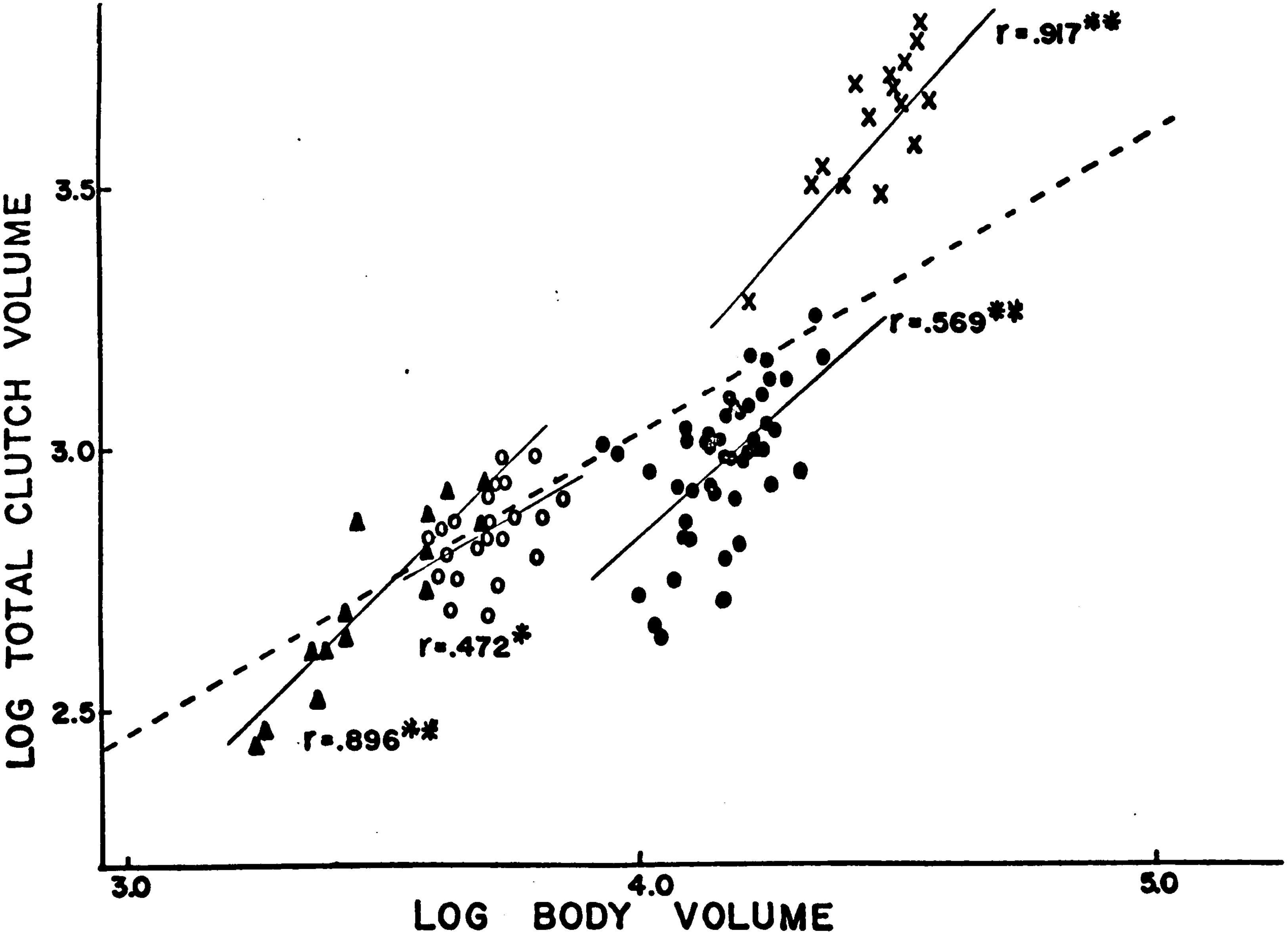

LOG TOTAL CLUTCH VOLUME
3.5
3.0
2.5
LOG BODY VOLUME
3.0
4.0
5.0
r=.917**
r=.569**
r=.472*
r=.896**

Figure 15. Log clutch size plotted against log body volume (mm^3) for four populations of Ambystoma. (Symbols as in figure 14). Dashed line is the model and is taken from figure 12. ** = $p \leq 0.01$.

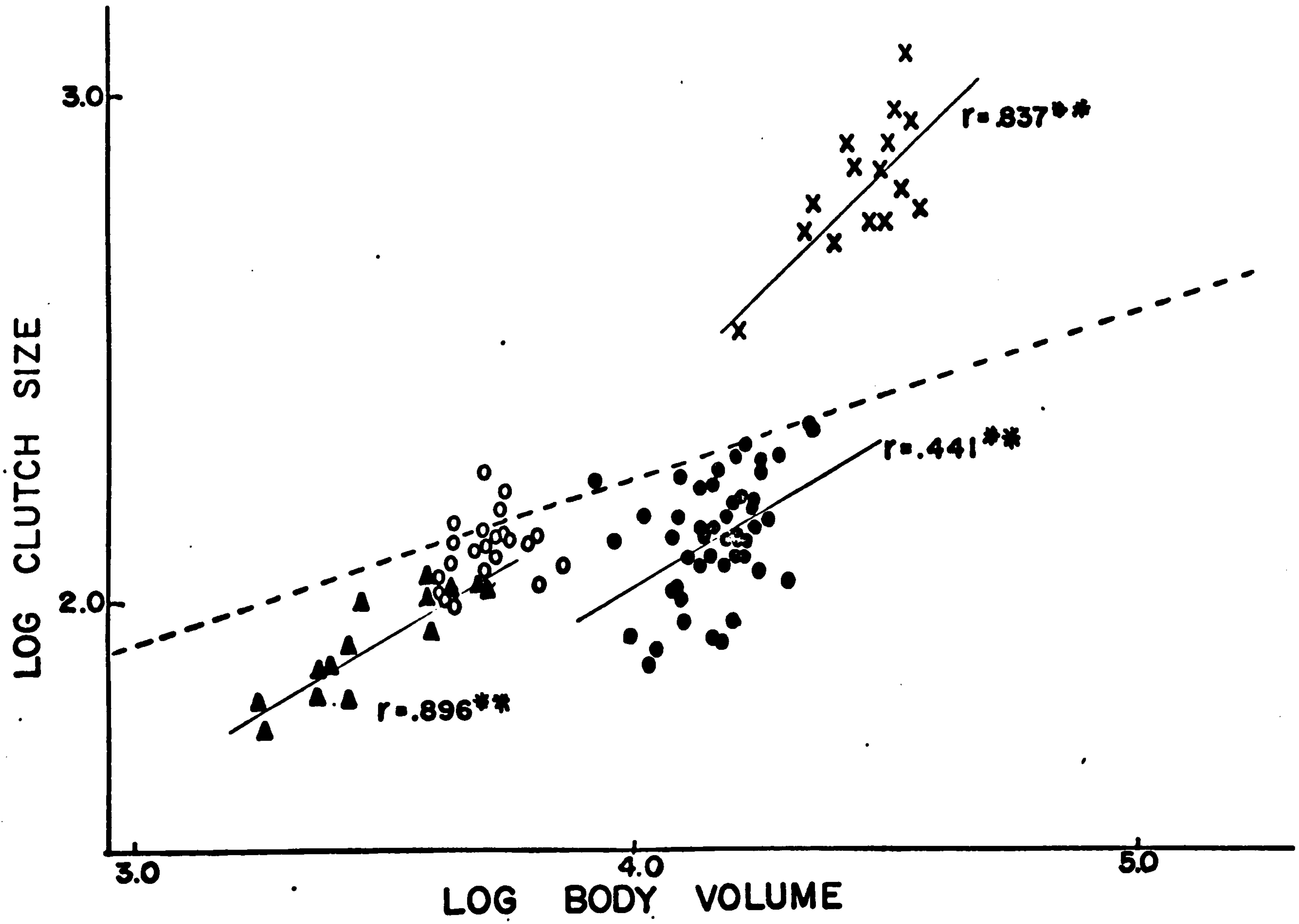
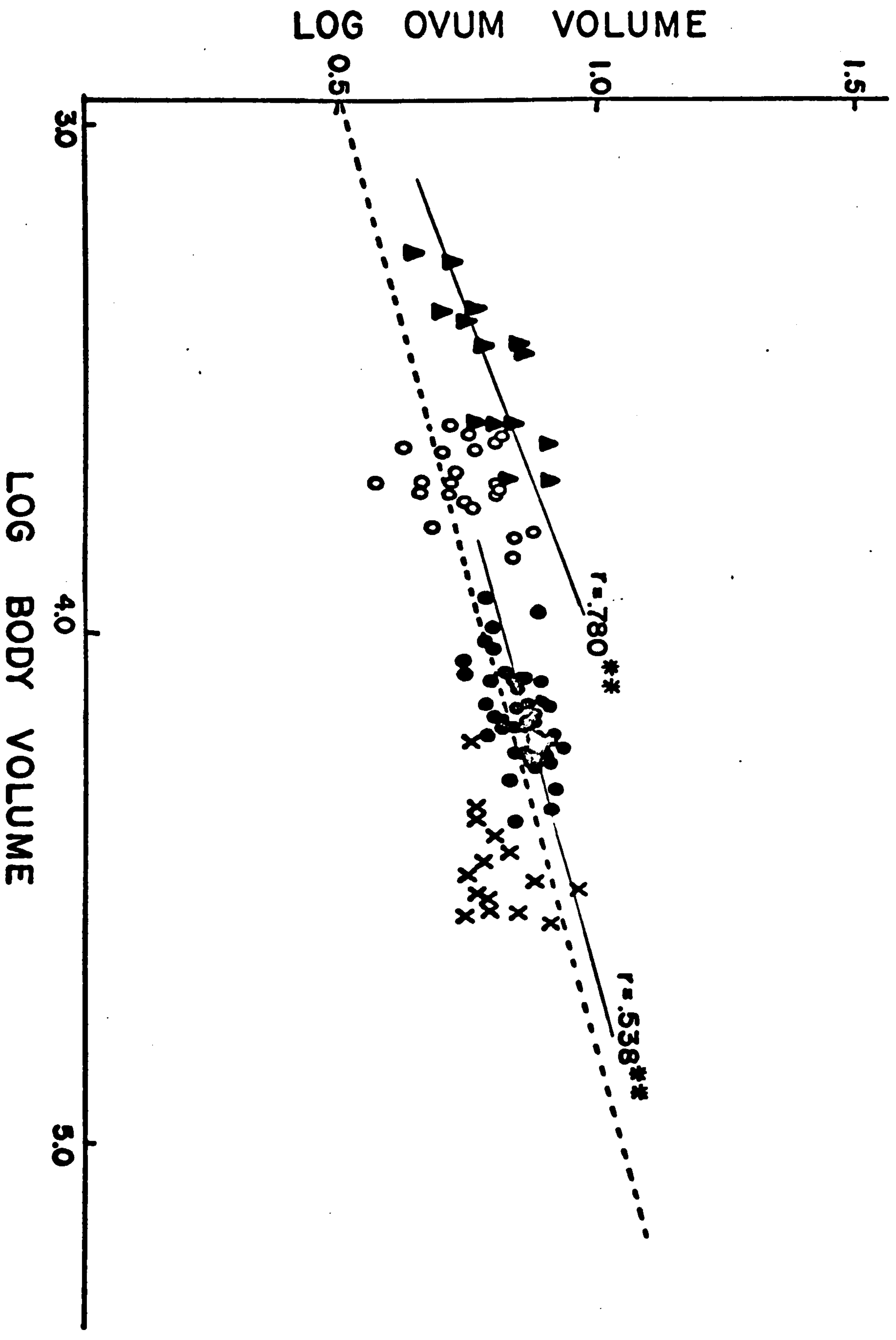

LOG CLUTCH SIZE
3.0
2.0
LOG BODY VOLUME
3.0
4.0
5.0
r=.837**
r=.441**
r=.896**

Figure 16. Log ovum volume (mm^3) plotted against log body volume (mm^3) for four populations of Ambystoma. (Symbols as in figure 14). Dashed line is the model and is taken from figure 13.

** = $p \leq 0.01$.


LOG OVUM VOLUME
0.5
1.0
1.5
LOG BODY VOLUME
3.0
4.0
5.0
r=.780**
r=.538**

by which population deviations can be discovered, or a generic model with which species can be compared (Tilley, 1968). Because of the present scarcity of such data, it is necessary to construct models inclusive of species and genera in order to obtain sufficient data points, and a useful one may be formed at the level of reproductive mode. As already mentioned, the clutch volume - body volume relationship is nearly identical regardless of reproductive mode or taxon. However, there is considerable modal variability in clutch size and ovum volume. Therefore, a model based on mode I salamanders will be used here for comparison with the populations of *Ambystoma*. (It should be realized that due to weak correlation, the slopes may not be as accurately determined as required for some purposes. However, the t-test that is employed on the deviations takes the variability into account.)

Figure 15 shows the relationship between egg number and body volume. Here it is seen that *A. opacum*-S.C. which had a predicted clutch volume, has fewer eggs than would be expected for a mode I salamander of the same size. *A. opacum*-N.J. is once again on the line. *A. maculatum*, which has a relatively small clutch volume, has fewer eggs than expected, while *A. tigrinum*, with a relatively large clutch volume, produces many more than the predicted number. When ovum volume is plotted against body volume (figure 16), it is seen that the means for all populations and species are similar despite large differences in body size (and actual significant differences in mean ovum volume). Thus, in reference to the model, *A. tigrinum* has relatively smaller ova, and *A. opacum*-S.C. relatively larger ones, than predicted for animals of their respective size. The general lack of correlation between ovum size and body size among these four populations speaks strongly for a conservative ovum size within the genus as a whole, possibly related to developmental modal constraints. In fact, all species of Ambystoma for which data have been taken (including the stream breeding A. rosaceum - Anderson, 1961) have ova of a similar size. (A. laterale may be an exception; Wilbur, 1977).

Within populations there are two types of relationships between clutch volume and body volume (table 2). In the A. tigrinum and A. opacum-S.C. populations there is a high positive correlation between the two variables. There is also a significant correlation in the other two populations, but the association is much weaker. (In A. opacum-N.J. this is partly because of the restricted range of body sizes.) Where the correlation is high the slopes are high; where they are weak, the slopes are diminished (a common bias of least - squares regression). The interesting point here is that despite overall interspecific negative allometry, intrapopulational slopes can be quite variable and in fact approach isometry.

The intrapopulational variability that is found in the clutch volume - body volume relationship can be explained on the basis of variability in the components of clutch volume. Thus, the high correlation observed in A. tigrinum is due to the variability in clutch size being highly correlated with that of body size while variability in egg size, which is not correlated with body size, has little effect on the differences in clutch volume among individuals. In A. opacum-S.C. the similarly high association between clutch volume and body size is due to changes in both clutch size and ovum size, both being significantly positively correlated with body size. The weak associations between clutch volume and body size in the *A. maculatum* and *A. opacum*-N.J. populations are due to weak but significant positive correlations in both components of clutch volume in the former case and to nonsignificant correlations of the components in the latter (tables 3 and 4).

THE OFFSPRING

The egg size data that were presented at the beginning of the results section can be used to provide clearer insight into the factors that regulate clutch size and total reproductive output from the point of view of the female parent (see above and discussion). It was also shown that this variability can result in some individuals in the population producing eggs that have a functional size (volume) double that of others. From the point of view of the offspring the all-encompassing question that needs to be asked is what are the implications of this large amount of variability on subsequent development and growth, and, by inference, on viability?

Material for growth

It might be presumed that all *Ambystoma* salamanders package an equivalent amount of material into a specific volume of egg so that ovum diameter or volume measurements would be a sufficient indicator of the size of an offspring's starting package. An analysis of variance of the differences in density of material (mgs. dry weight/ vol.) among four populations (*A. opacum*-Ga. replaces *A. opacum*-S.C. in the analysis) indicates that there are significant differences ($F = 5.07$, $DF = 3,29$; $p \leq 0.01$) with *A. tigrinum* producing eggs with the most material in them (0.513 mgs./mm^3, SD = 0.0433) and *A. maculatum* producing ova with the least material per unit volume (0.445 mgs./mm^3, SD = 0.0295). These differences would obviously result in an overestimation of the material provided to individual offspring in *A. maculatum* relative to *A. tigrinum*. It therefore appears that egg diameters or volumes by themselves cannot be used reliably to describe the commitment of materials to new offspring.

The significant differences that were found within and among populations with reference to ovum diameters and volumes are maintained in reference to dry weight (table 5) but the differences between the populations have changed. For example, from figure 6 it is seen that *A. opacum*-Ga. produces the largest ova; *A. tigrinum* and *A. maculatum* produce smaller ova of nearly equal size; and *A. opacum*-N.J. produces the smallest ova. The dry weight data (table 5), indicate that *A. opacum*-Ga. and *A. tigrinum* produce the heaviest ova and *A. maculatum* and *A. opacum*-N.J. produce equally sized lighter ova. Dempster (1930 and 1933) reported *A. maculatum* eggs (from Michigan) having dry weights of approximately 2.34 and 2.45 mgs. Hopkins and Handford (1943) reported *A. maculatum* eggs (mixed localities) with dry weights of 2.67 - 3.29 mgs. and *A. tigrinum* eggs (mixed localities) with dry weights of 2.48 - 2.93 mgs.

Energy for growth

Ovum volume is not a sufficient measure of the weight of materials contained in the egg. Similarly, the variability that exists in the dry weight of ova may not completely describe differences in energy commitment by individual parents and in energy available to siblings since the dry weight-specific energy contents may differ. This has been shown to be the case in lizards (Tinkle and Hadley, 1975) and in frogs Crump and Kaplan, in prep.). Because of insufficient materials, ash content of the eggs in *Ambystoma opacum* was not measured. However, there were no significant differences in the ash contents of *A. maculatum* and *A. tigrinum* ova (F = 2.55, DF = 1,17) so one average ash

Table 5. Non-parametric analysis of variance (Kruskal-Wallis test) in dry weights of ova of four populations of *Ambystoma*.

population	mean(SD) (mgs.)	range (mgs.)	N	χ^2	p
A. opacum$_{Ga.}$	3.62(0.233)	3.29-3.82	7	35.3	0.005
A. opacum$_{N.J.}$	2.73(0.475)	2.32-3.61	5	25.0	0.005
A. maculatum	2.78(0.321)	2.12-3.34	9	86.4	0.005
A. tigrinum	3.53(0.677)	2.77-4.75	8	116.4	0.005
Among pops.	---	---	4	17.5	0.005

value of 5.33% (SD = 0.257) seems to apply to all these ova. This value is close to the value of 3.67% found by Crump and Kaplan (in prep.) for the eggs of tree frogs and to the 4.3% value reported by Boyd and Goodyear (1971) for a variety of amphibians, but differs considerably from the 17% value reported by Ochs (1970) for Bufo americanus and the 0.55% value reported for Desmognathus ochrophaeus (Fitzpatrick, 1973). Because ash values for ova among the Ambystomas studied here are considered constant, the dry weight-specific energy contents reported will not be corrected for ash. Individual mean values and population mean values are shown in figure 17. The difference between the two populations of A. opacum and the other two species is striking. Not only are the populations significantly different but individual clutches within a population can also differ significantly from each other. This is made clear by a nested analysis of variance presented in table 6. The outcome of not considering these differences would be that energy content of A. opacum ova would be underestimated relative to the ova of A. maculatum and A. tigrinum and a similar bias would be introduced at the level of the individual.

Variability in both egg densities and dry weight-specific caloric content are taken into account if the total energy contained within the egg is considered. Mean values for individuals and the four populations are presented in figure 18. Non-parametric statistics (Kruskal-Wallis test; table 7) reveal that individual females within a population produce eggs that contain different amounts of energy and that there is significant variability among populations as well. (Because of the differences in material density and weight-specific energy content, neither dry weight of the clutch nor total energy of the clutch reflect

Figure 17. Dry weight-specific caloric content of ova of four populations of *Ambystoma*. Closed circles = mean values for specific females. Open circles = grand mean for population. A = *A. opacum*$_{Ga.}$; B = *A. opacum*$_{N.J.}$; C = *A. maculatum*; D = *A. tigrinum*.

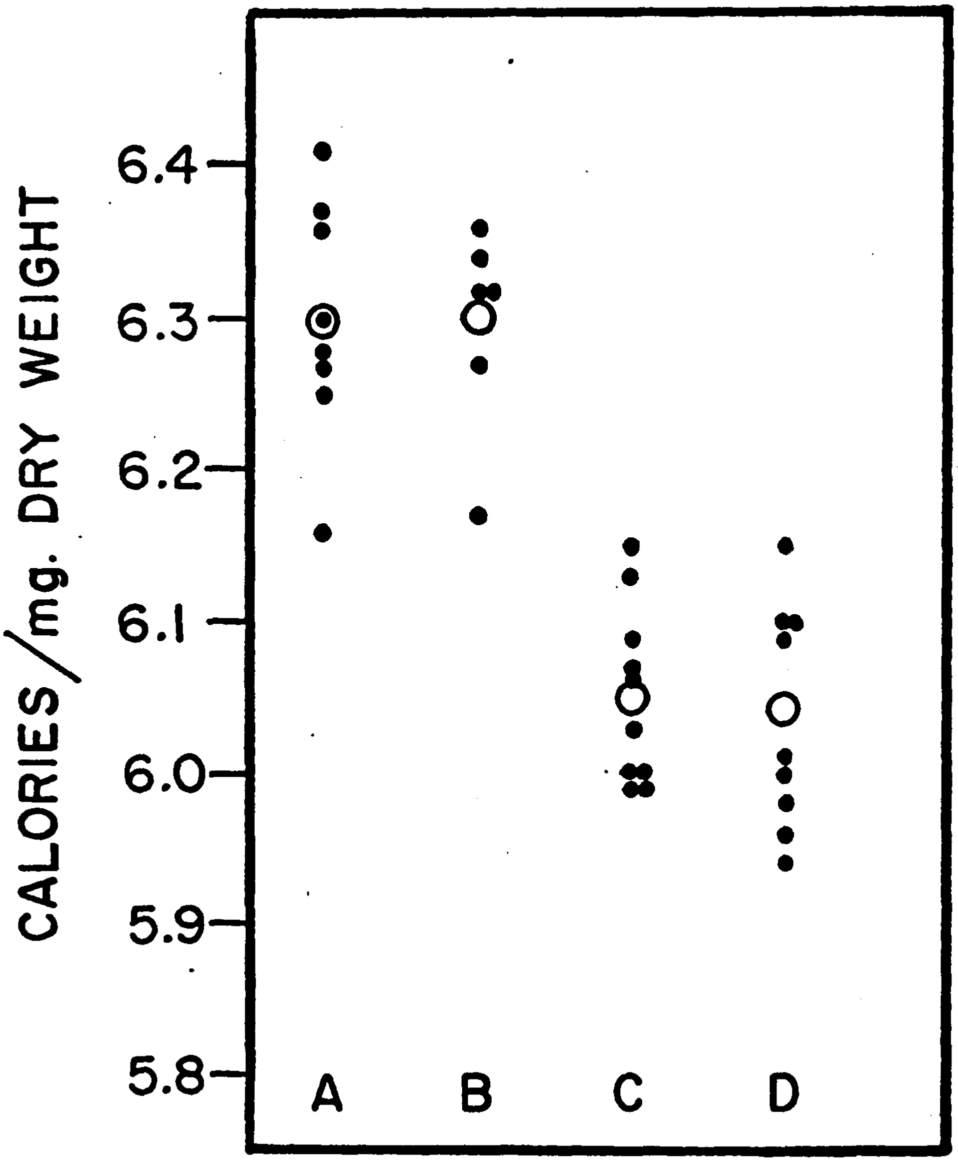

CALORIES/mg. DRY WEIGHT
6.4
6.3
6.2
6.1
6.0
5.9
5.8
A
B
C
D

Table 6. Nested analysis of variance in dry weight-specific caloric content of ova of four populations of *Ambystoma*.

source	df	SS	MS	F	P
populations	3	3.69	1.228	29.2	0.001
individuals	29	1.25	0.043	1.7	0.05
determinations	259	6.65	0.026		
total	291	11.59			

Figure 18. Total calories contained in the ova of individuals of four populations of Ambystoma. (Symbols as in figure 17).

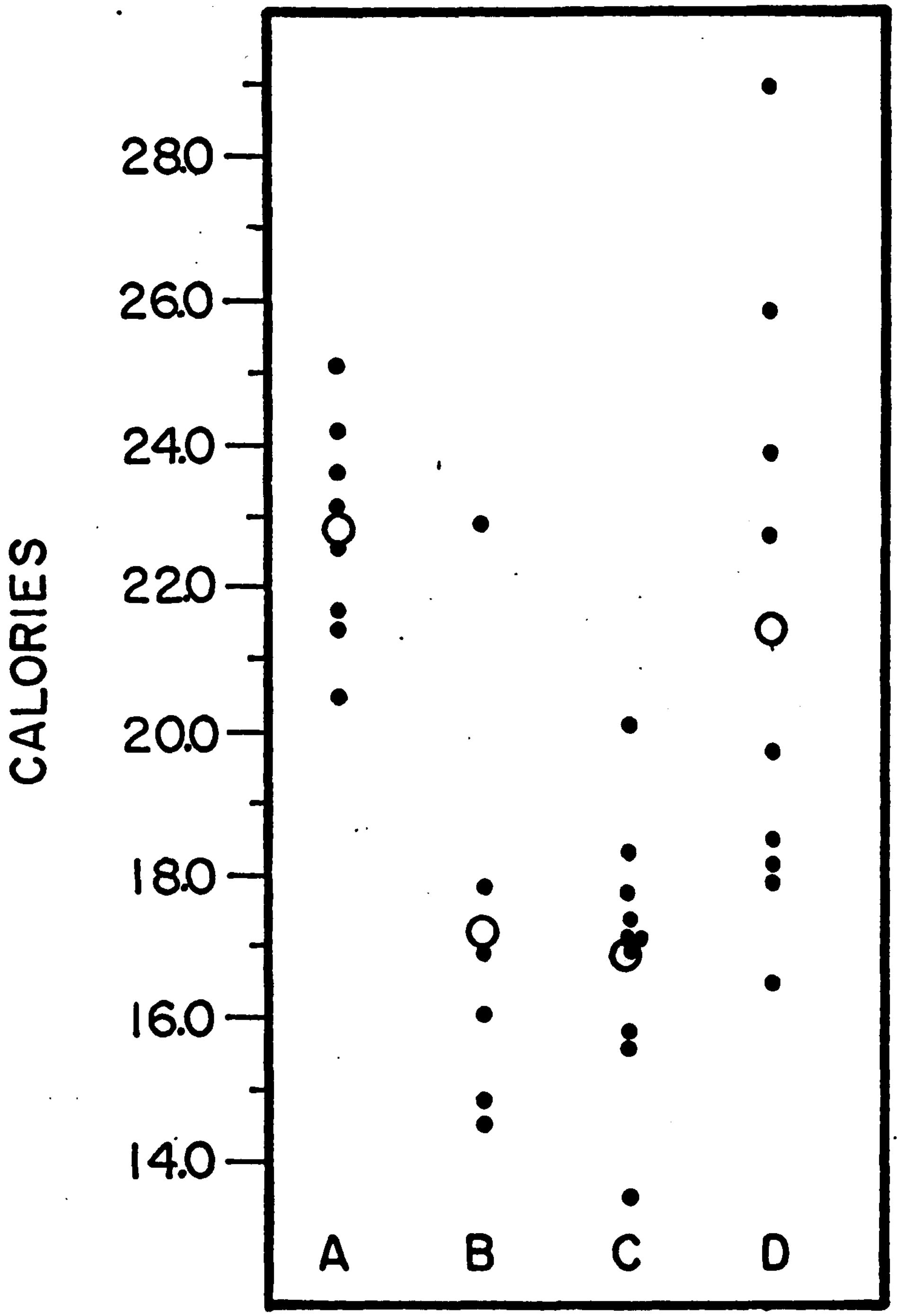

CALORIES
28.0
26.0
24.0
22.0
20.0
18.0
16.0
14.0
A
B
C
D

Table 7. Non-parametric analysis of variance (Kruskal-Wallis test) in total caloric content of ova of four populations of *Ambystoma*. (Data from figure 18).

population	N	χ^2	P
A. opacum Ga.	8	29.4	0.005
A. opacum N.J.	6	24.2	0.005
A. maculatum	10	76.9	0.005
A. tigrinum	9	87.5	0.005
among pops.	4	16.8	0.005

body size constraints to the extent that volume does).

The amount of variability that exists within one population is most extreme in Ambystoma tigrinum, where eggs of one female contained an average of 16.2 calories while the eggs of another female contained 29.0 calories. The offspring of the former female begin life with almost half the energy of the latter:

The inter- and intrapopulational variability that exists in ovum density and dry weight-specific energy content (that make both ovum volume and dry weight unsatisfactory predictors of the energy contained in ova) could interact in such a way that energy density of an egg (energy/mm^3) could be constant for these species. However, as the data presented in table 8 show, there is significant variability between populations in this variable as well. The implications of energy density variability (and the general relationship between ovum volume and energy) are illustrated by the positions of individual values and population clusters relative to the regression line of all the data combined (figure 19). Clearly, there is a need to evaluate the influence of the large amount of variability in energy and body cavity space committed to individual offspring by different females within a population.

Developmental rate to the hatching stage

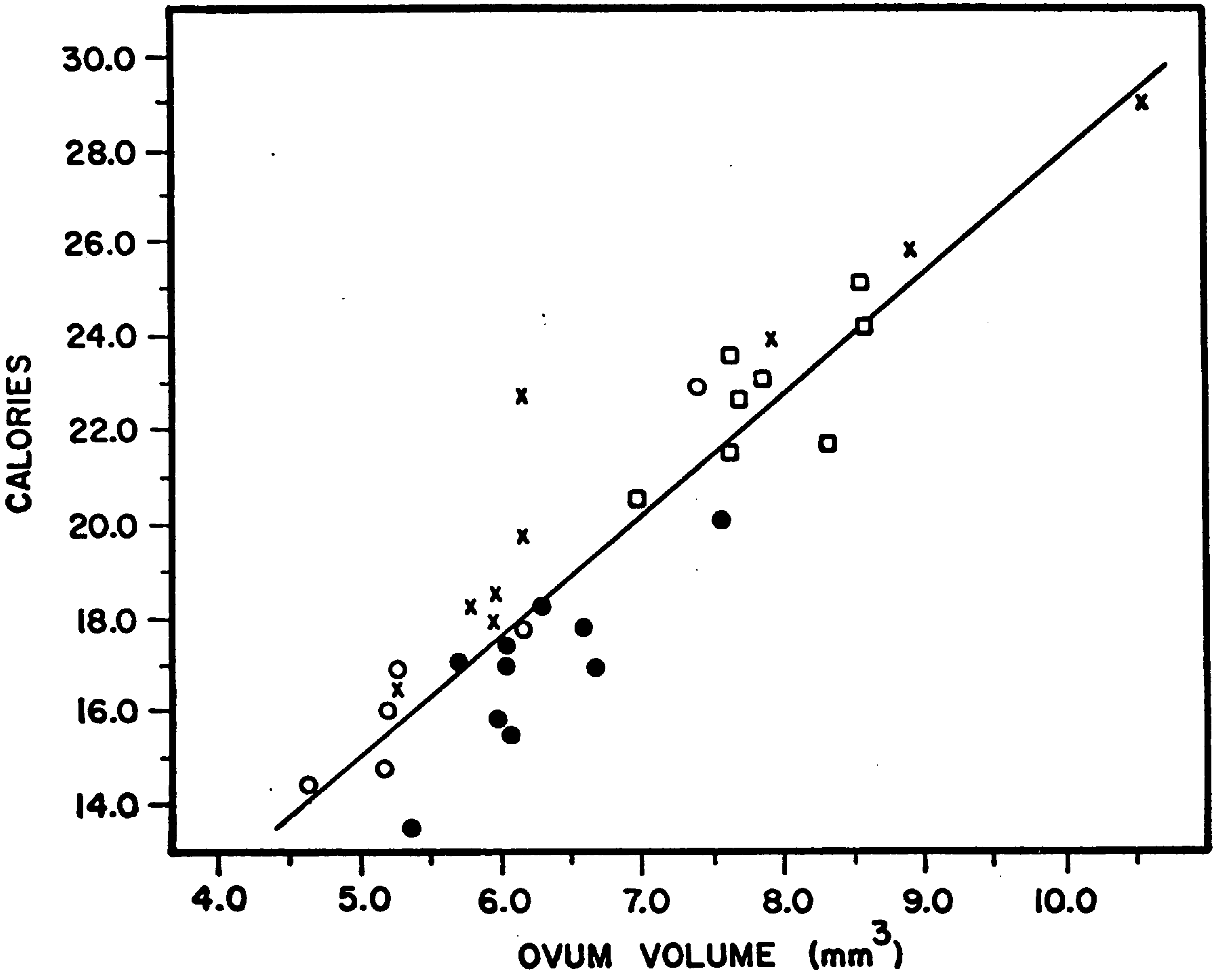
From strictly interspecific data numerous authors (see discussion) have suggested a negative correlation between ovum size and developmental rate. If this relationship has its basis in a direct cause-and-effect phenomenon (e.g., larger ova cleave less readily) then this should be reflected at the populational level. The time required to develop from gastrula to hatching for the four populations grown under

Table 8. Mean values and analysis of variance in energy density (calories/mm^3) of ova of four populations of *Ambystoma*.

population	*A. opacum*$_{\text{Ga.}}$	*A. opacum*$_{\text{N.J.}}$	*A. maculatum*	*A. tigrinum*
mean(SD)	2.88(0.142)	3.05(0.144)	2.72(0.171)	3.10(0.265)
N	8	6	10	9

source	df	SS	MS	F	P
among groups	3	0.782	0.261	7.07	0.005
within groups	29	1.069	0.037		
total	32	1.852			

Figure 19. Calories per ovum regressed on ovum volume. Points are mean values for ova of specific females. Open squares = A. opacum$_{Ga.}$; open circles = A. opacum$_{N.J.}$; closed circles = A. maculatum; crosses = A. tigrinum.


30.0
28.0
26.0
24.0
22.0
20.0
18.0
16.0
14.0
CALORIES
4.0
5.0
6.0
7.0
8.0
9.0
10.0
OVUM VOLUME (mm^3)

three temperature regimes is shown in figure 20. It should be noted that A. tigrinum has an extremely fast developmental rate relative to the other two species at the coldest temperature and that there is significant interpopulational variability at the colder temperature between the two A. opacum populations (t_s =5.55, df =1,10, $p \leq 0.001$). A. opacum need not necessarily have a slower developmental rate than A. maculatum as has been reported in the literature for development at 20°C (Moore, 1939). In addition it should be noted that developmental times in the alternating environment are not midway between the two extreme environments, but more closely approximate the shorter developmental times of the warmer temperature.

Correlations were calculated between ovum volume, total energy content, dry weight, material density, energy density, dry weight-specific energy content and time between gastrula and hatching in all four populations at the three temperature conditions (excluding the cycling temperature for A. opacum-Ga.). There is little indication of any effects of these egg parameters on the time required to reach the hatching stage (table 9). The several significant correlations that do appear in the table are due to two individuals in the case of A. maculatum at 10°C and to one individual in A. opacum-N.J. In addition, the latter correlations are opposite in sign to the former. It therefore seems reasonable to conclude that neither egg size nor energy affect early embryonic developmental rates in any readily discernable way and that the interspecific correlations reported in the literature are due to other factors.

Hatchling size

The influence of ovum volume variability on hatchling total length

Figure 20. Time in hours between gastrula and hatching for four populations of *Ambystoma* at three experimental temperatures. Each point is the value for the embryos of an individual female. The first group developed at a constant 10° C; the second at a constant 20° C; the third in an environment that alternated between 10° and 20° C. A = *A. opacum*$_{Ga.}$; B = *A. opacum*$_{N.J.}$; C = *A. maculatum*; D = *A. tigrinum*.

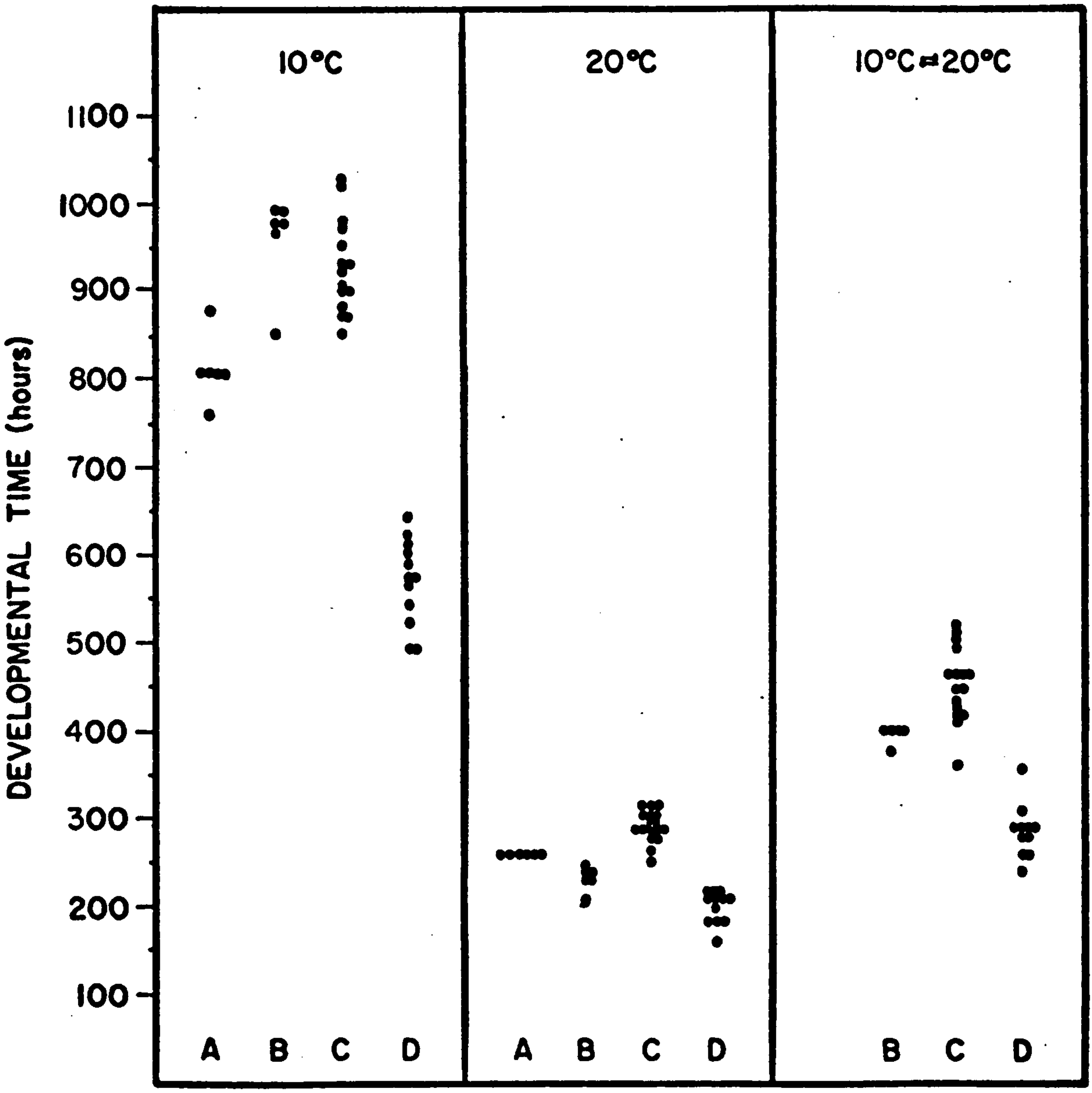

10°C
20°C
10°C⇌20°C
DEVELOPMENTAL TIME (hours)
1100
1000
900
800
700
600
500
400
300
200
100
A
B
C
D
A
B
C
D
B
C
D

Table 9. Product-moment correlation coefficients between various ovum parameters (ovum volume, dry weight, total calories, calories/mg., calories/mm^3, mgs./mm^3) and developmental time between gastrula and hatching for four populations of *Ambystoma* at three temperature regimes. (* = $p \leq 0.05$; ** = $p \leq 0.01$; sample sizes in parentheses).

population	ovum volume	dry weight	total cal	cal/mg	cal/mm^3	mgs/mm^3
(10°C)						
*A. opacum*Ga.	-0.33	-0.09	-0.17	-0.30	0.23	0.26
	(6)	(6)	(6)	(6)	(6)	(6)
*A. opacum*N.J.	0.07	-0.08	-0.09	-0.26	-0.66	-0.53
	(6)	(6)	(6)	(6)	(6)	(6)
A. maculatum	-0.07	0.92**	0.85*	-0.91**	-0.18	0.33
	(15)	(7)	(7)	(7)	(7)	(7)
A. tigrinum	0.47	0.40	0.36	-0.40	0.14	0.07
	(12)	(7)	(7)	(7)	(7)	(7)
(20°C)						
*A. opacum*Ga.	0.00	0.00	0.00	0.00	0.00	0.00
	(6)	(6)	(6)	(6)	(6)	(6)
*A. opacum*N.J.	-0.06	-0.06	-0.02	0.77	0.14	-0.02
	(6)	(6)	(6)	(6)	(6)	(6)
A. maculatum	0.15	0.19	0.21	-0.15	0.20	0.21
	(17)	(9)	(9)	(9)	(9)	(9)
A. tigrinum	-0.06	0.56	0.53	-0.42	-0.22	-0.22
	(12)	(7)	(7)	(7)	(7)	(7)
(10°⇄20° C)						
*A. opacum*Ga.	-----	-----	-----	-----	-----	-----
*A. opacum*N.J.	-0.84	-0.89*	-0.88*	-0.43	-0.45	-0.32
	(5)	(5)	(5)	(5)	(8)	(5)
A. maculatum	0.59	0.13	0.20	0.17	0.17	0.06
	(16)	(8)	(8)	(8)	(8)	(8)
A. tigrinum	-0.35	-0.25	-0.26	0.08	0.10	0.15
	(11)	(4)	(4)	(4)	(4)	(4)

among females of the four populations is presented in figure 21. There are positive correlations between the two variables at all experimental temperatures and in all populations (except in A. opacum-Ga. where ovum volume variability is highly truncated). The relationship has been observed interspecifically and interpopulationally in a variety of amphibian groups (see discussion) but has never previously been demonstrated intrapopulationally.

The effect of temperature on hatchling size which appears in figure 21 may be explained by the relationship presented in figure 22. From the latter figure it is seen that among all individuals in all populations the more the Q_{10} deviates from approximately three-fold with regard to developmental time between 10°C and 20°C the greater the affect on hatchling size. Q_{10}'s less than 3.0 (mostly in A. tigrinum) are associated with hatchlings that are larger when they develop at the warmer temperatures while Q_{10}'s greater than 3.0 (A. opacum-N.J.) are associated with hatchlings that are smaller when they develop at warmer temperatures. In other words, the greater the inhibition of developmental rate by diminishing temperature, the larger the resultant hatchling at that colder temperature. The alternating temperature regime results in hatchlings that are generally intermediate in size.

When total calories or dry weight contained in hatchlings is used as the measure of larval size, even stronger correlations exist than those between ovum volume and hatchling total length (table 10). No relationship exists, however, between the temperature at which the embryos develop and the energy they contain at the hatching stage. (This is surprising in light of the effects of temperature on developmental rate and hatchling length presented above.) Furthermore, neither

Figure 21. Total length at hatching plotted against ovum volume for four populations of Ambystoma at three temperature regimes. a = A. opacum$_{Ga.}$; b = A. opacum$_{N.J.}$; c = A. maculatum; d = A. tigrinum. crosses = development at 20°C; open circles = development at $10^{\circ} \rightleftarrows 20^{\circ}$ C; closed circles = development at 10°C. r = product-moment correlation coefficients. * = $p \leq 0.05$; ** = $p \leq 0.01$.

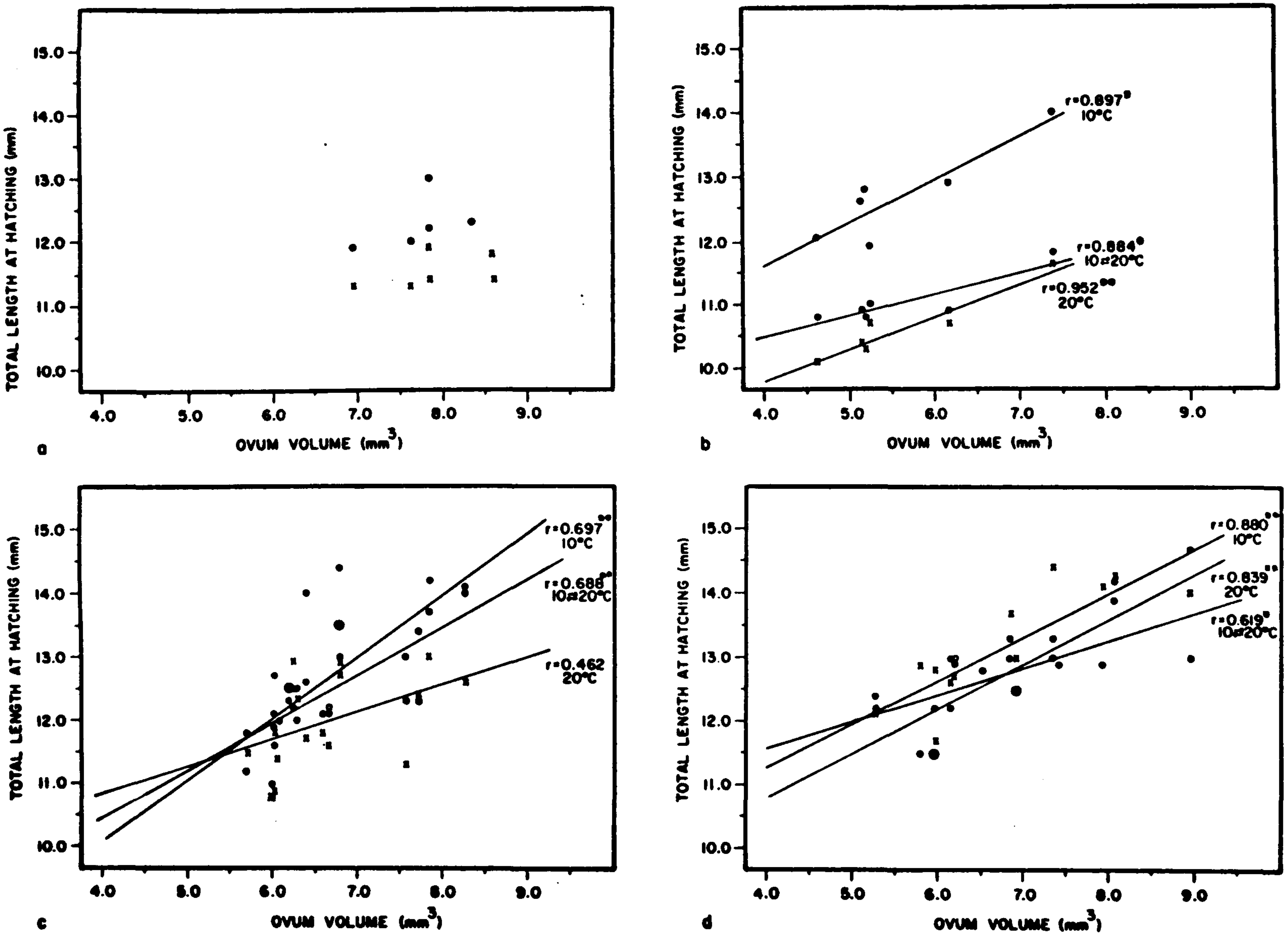

TOTAL LENGTH AT HATCHING (mm)
15.0
14.0
13.0
12.0
11.0
10.0
4.0
5.0
6.0
7.0
8.0
9.0
OVUM VOLUME (mm^3)
a
b
r=0.897*
10°C
r=0.884*
10≠20°C
r=0.952**
20°C
c
r=0.697**
10°C
r=0.688**
10≠20°C
r=0.462
20°C
d
r=0.880**
10°C
r=0.839**
20°C
r=0.619*
10≠20°C

Figure 22. Q_{10} in developmental time between 10° and 20° C plotted by the ratio of total hatchling length at 20° C to that at 10° C. Dashed line indicates equal hatchling sizes at the two temperatures. open squares = A. opacum$_{Ga.}$; open circles = A. opacum$_{N.J.}$; closed circles = A. maculatum; crosses = A. tigrinum.

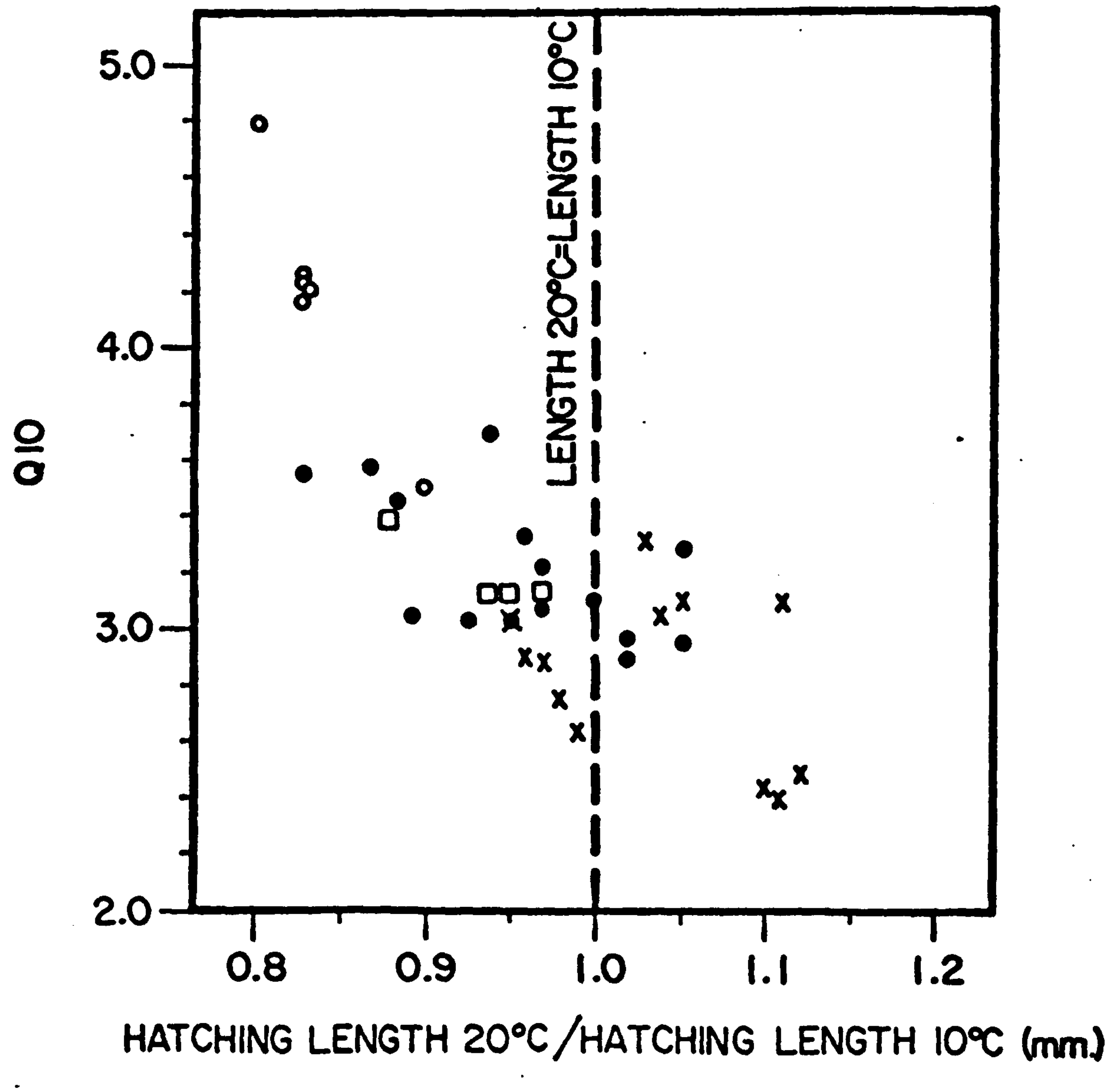

5.0
4.0
3.0
2.0
Q10
LENGTH 20°C=LENGTH 10°C
0.8
0.9
1.0
1.1
1.2
HATCHING LENGTH 20°C/HATCHING LENGTH 10°C (mm)

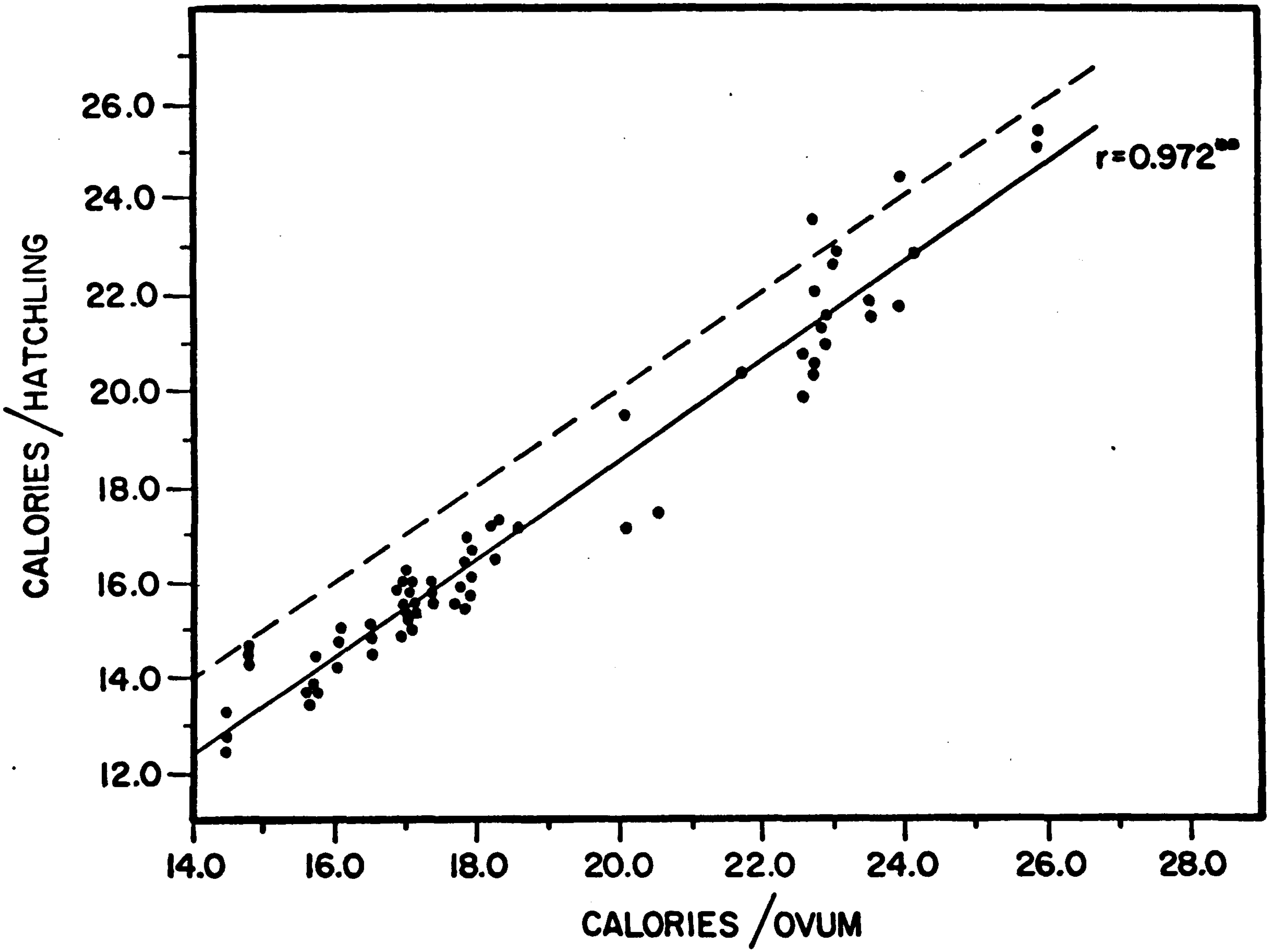
Table 10. Product-moment correlation coefficients between total calories contained in ova and total calories contained in hatchlings for four populations of *Ambystoma* at three temperature regimes. (* = $p \leq 0.05$; ** = $p \leq 0.01$; sample sizes in parentheses.)

population	10° C	20° C	10°⇄20° C
A. opacum$_{\text{Ga.}}$	0.934* (5)	0.729 (4)	-----
A. opacum$_{\text{N.J.}}$	0.970** (6)	0.980** (6)	0.989** (6)
A. maculatum	0.988** (6)	0.967** (9)	0.813** (9)
A. tigrinum	0.985** (7)	0.992** (5)	0.999** (4)

temperature nor size of the initial investment has an effect on the amount of energy used to reach the hatching stage. All the embryos of all four populations at all temperatures use approximately 1.45 calories (SD = 0.781) to reach the hatching stage. This is roughly equivalent to an average of 7.0% of the initial caloric content of the ovum. The relationship between hatchling calories and ovum calories is therefore a line which is approximately parallel to a line of perfect conversion of egg calories into hatchling calories but whose elevation is diminished by the number of calories generally used (figure 23). Individuals that begin life with a smaller amount of energy require as much energy to reach the hatching stage as those that start out with more energy. In _A. tigrinum_ for example, this results in larger individuals using 5.6% of their initial energy while the smaller ones use 8.8%.

The increase in total length of the hatchlings at colder temperatures is not associated with any energetic parameter. It doesn't cost more to become larger nor does the larger hatchling contain more energy. It has been reported that organisms grown at colder temperatures are larger at equivalent developmental stages (see for example Uhlenhuth, 1919; Gray, 1928; Lock and McLaren, 1970), and that _Ambystoma_ embryos show some differences with respect to shape during early developmental stages (Dempster, 1930). It would ordinarily be assumed that such differences are accompanied by differences in energy utilization, but the data here show that this is not the case. In all four populations, however, the dry weight-specific caloric content of hatchlings that developed at the colder temperature is lower. The relationship is significant in two out of the four populations at $p \leq 0.05$, and must be

Figure 23. Calories contained in hatchlings plotted against calories contained in ova for individuals of all four Ambystoma populations combined. The dashed line shows 100% efficiency of conversion of ovum energy to hatchling energy. r = product-moment correlation coefficient; ** = $p \leq 0.01$.

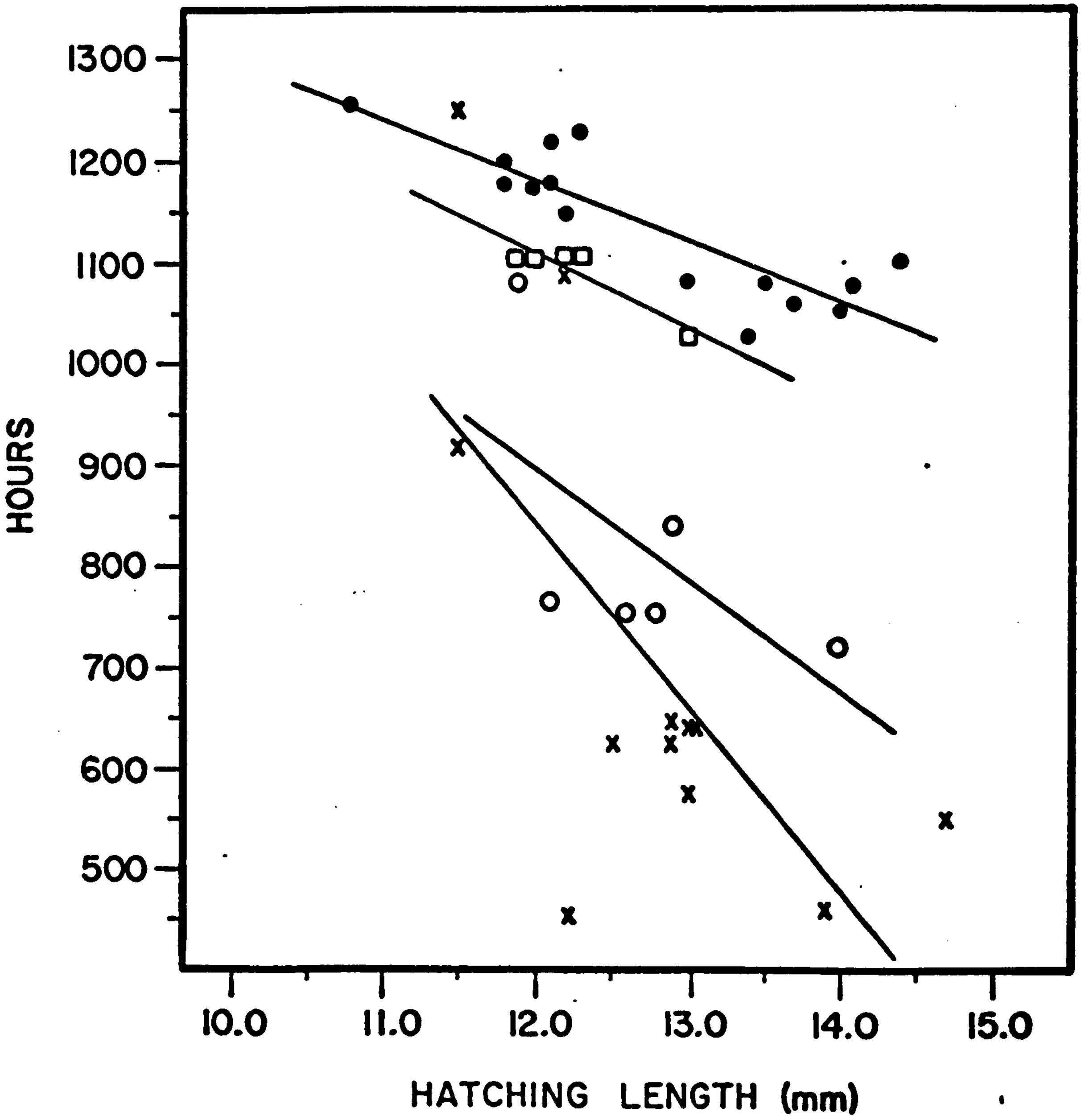

r=0.972**
CALORIES/HATCHLING
26.0
24.0
22.0
20.0
18.0
16.0
14.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
28.0
CALORIES/OVUM

due to differential usage of carbohydrate, fat and protein, which in fact has been shown to occur in A. mexicanum embryos that develop at different temperatures (Løvtrup, 1959). Several explanations therefore are available for why larger hatchlings have not used more energy to get larger. The conversion of high energy fat into hatchling tissue may occur with higher efficiency at colder temperatures. Alternatively, the water content of embryos may vary with temperature (Tuft, 1962) or the shape of the embryos (longer and thinner) may vary. These three alternatives cannot be distinguished at this time.

Developmental rate to the feeding stage

Even though the data show that neither size nor energy content of an offspring affects the time required for hatching, these factors may yet exert an influence on subsequent developmental time, especially since they so strongly affect the size of the hatchling. This indeed has been found to be the case. All correlations between hatchling total length and the time required for the hatchling to reach the feeding stage (for all populations at all temperatures) are negative. Most with sufficiently large samples are significant at the 0.05 level. The importance of the relationship is demonstrated by an examination of figure 24 which shows the negative relationships for the four populations at 10° C. From the figure it can be seen that embryos that hatch at the same time but at different sizes can vary considerably in the time required to reach the feeding stage, and a large component of this variability is due to hatchling size. At 10°C for example, the variability amounts to 3.5 days difference among the embryos of different females from the A. opacum-Ga. population. The difference amounts

Figure 24. Time in hours between hatching and feeding stages plotted against hatchling total length for development at 10°C. Open squares = *A. opacum*$_{Ga.}$; open circles = *A. opacum*$_{N.J.}$; closed circles = *A. maculatum*; crosses = *A. tigrinum*.

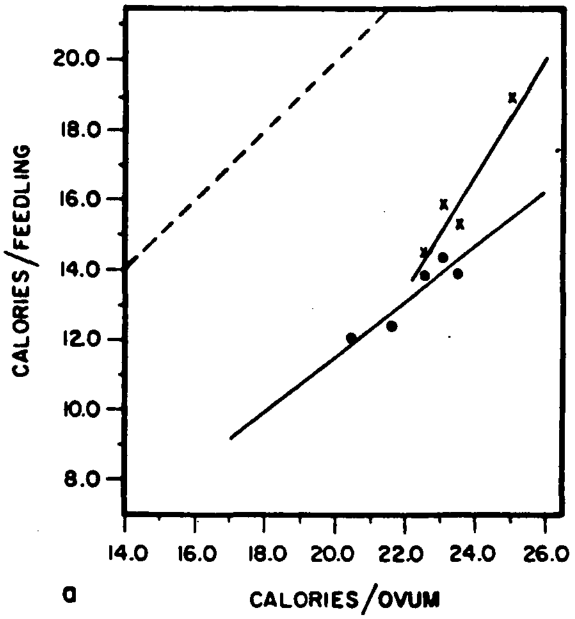

1300
1200
1100
1000
900
800
700
600
500
HOURS
10.0
11.0
12.0
13.0
14.0
15.0
HATCHING LENGTH (mm)

to 9 days in A. maculatum, 2 weeks in A. opacum-N.J., and over 1 month in A. tigrinum. Since the feeding stage is the first opportunity for the offspring to begin utilizing an external energy source, these differences are of critical importance and will be related to other results (see below).

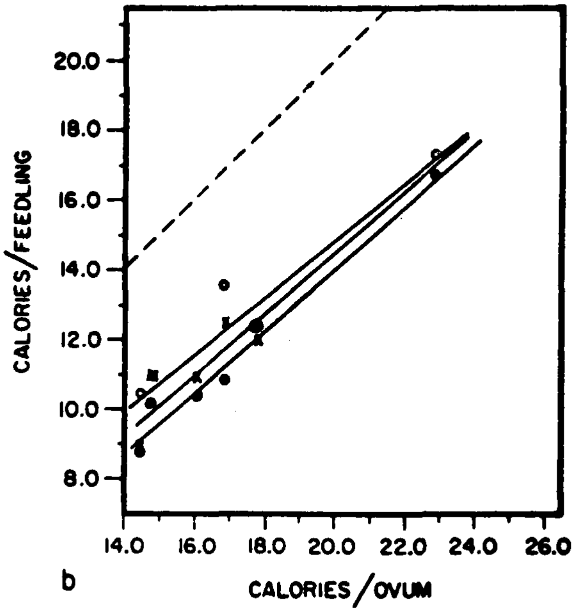
Larval respiratory rate (constant temperatures)

The effect of hatchling length on developmental time to feeding can be explained by the relationship between hatchling length and the rate at which the embryos metabolize energy. As suggested by figure 25 there is generally little relationship between the calories per ovum (which is highly correlated with hatchling size; see figure 23) and the amount of energy used to become a feedling. If the variability in surface area of a hatchling (presumed to be proportional to total length) is positively associated with respiratory rate (Davison, 1955), then the decrease in developmental time between hatching and feeding, associated with increased larval size, is expected. Figures 26 and 27 show the relationship between total oxygen consumption and hatchling length in A. tigrinum and A. maculatum for individual hatchlings at two constant temperatures. The regression equations in these figures can be used to predict the oxygen consumption rates of larvae of different sizes (from specific females). The microliters of oxygen consumed per hour can be converted to heat liberated per hour by using an oxycaloric coefficient of 4.7×10^{-3} cal/ul O_2 at STP (standard temperature and pressure; Kleiber, 1961). This value is based on the combustion of fat which is most appropriate for larvae at these stages of development (Løvtrup, 1959). Since the amount of energy required to develop from the

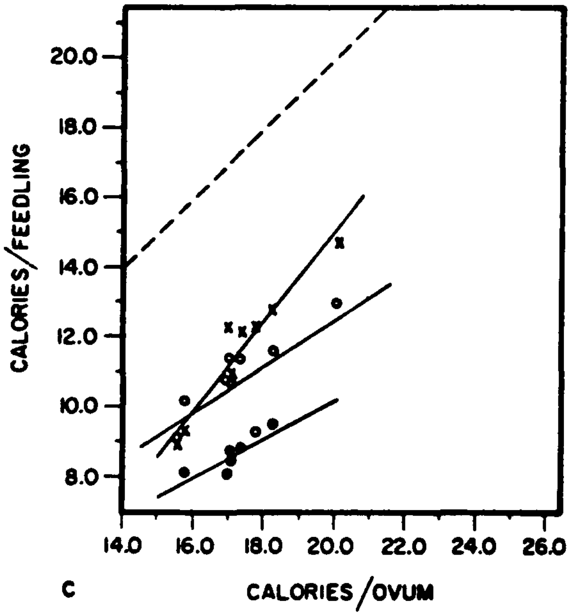
Figure 25. Calories contained in feedlings plotted against calories contained in ova. The dashed line shows 100% efficiency of conversion of ovum energy into feedling energy. a = A. opacum$_{Ga.}$; b = A. opacum$_{N.J.}$; c = A. maculatum; d = A. tigrinum. Closed circles = development at 10°C; open circles = development at 10°± 20° C; crosses = development at 20°C.


CALORIES/FEEDLING
8.0
10.0
12.0
14.0
16.0
18.0
20.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
CALORIES/OVUM

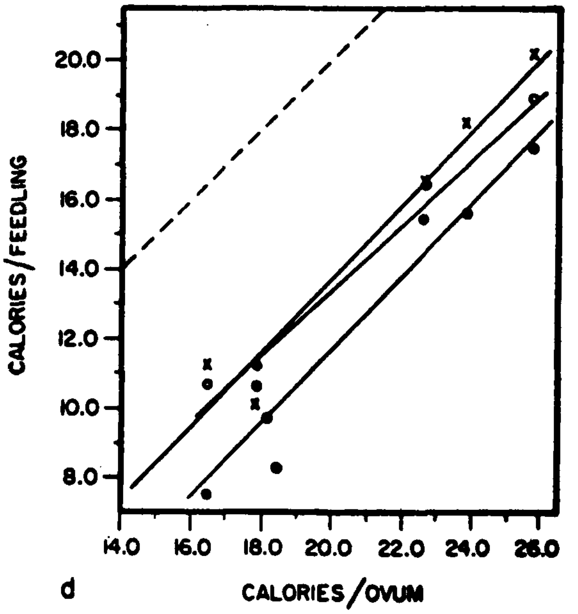
a


CALORIES/FEEDLING
8.0
10.0
12.0
14.0
16.0
18.0
20.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
CALORIES/OVUM

b


CALORIES/FEEDLING
8.0
10.0
12.0
14.0
16.0
18.0
20.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
CALORIES/OVUM

c


CALORIES/FEEDLING
8.0
10.0
12.0
14.0
16.0
18.0
20.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
CALORIES/OVUM

d

Figure 26. Oxygen consumption rates (microliters per hour per individual STP) plotted against hatchling length for *Ambystoma tigrinum* hatchlings at two constant temperatures.

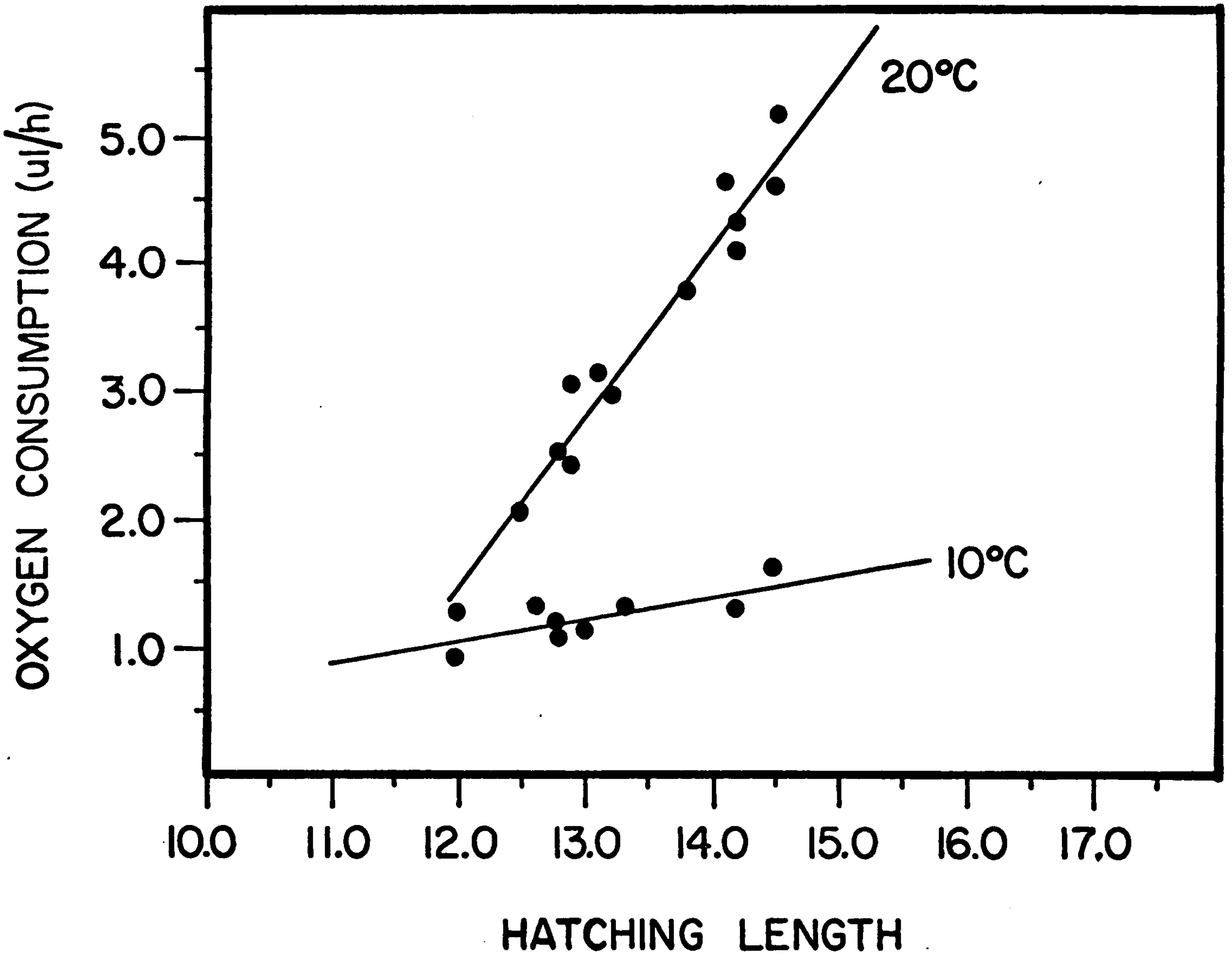
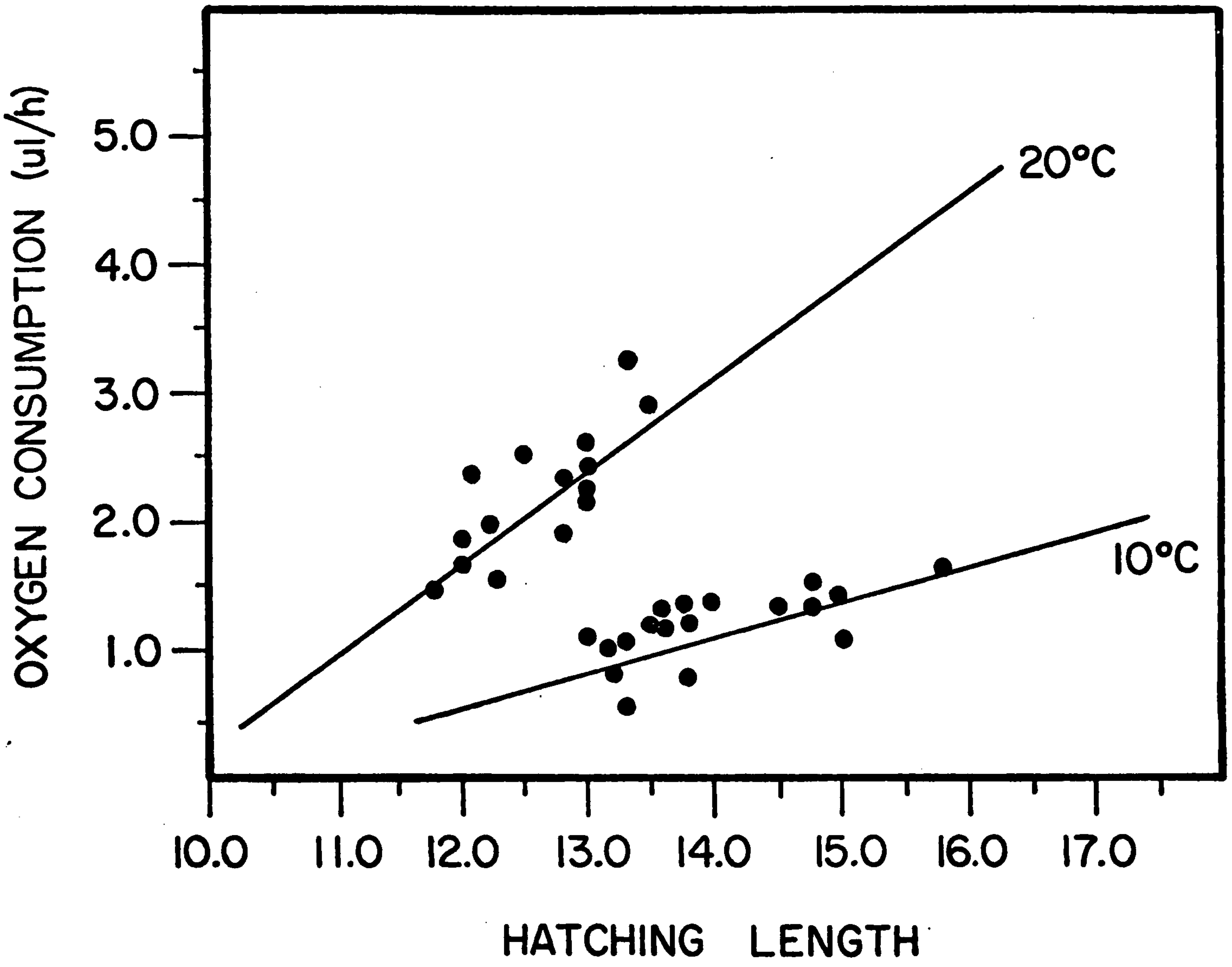

OXYGEN CONSUMPTION (ul/h)
1.0
2.0
3.0
4.0
5.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
HATCHING LENGTH
20°C
10°C

Figure 27. Oxygen consumption rates (microliters per hour per individual STP) plotted against hatchling length for *Ambystoma maculatum* hatchlings at two constant temperatures.


OXYGEN CONSUMPTION (ul/h)
5.0
4.0
3.0
2.0
1.0
20°C
10°C
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
HATCHING LENGTH

hatching to the feeding stage has been determined calorimetrically, the total number of hours required to liberate the appropriate number of calories via respiration can be calculated. The resulting negative correlations that exist between hatchling length and hours between hatching and feeding (table 11) are then seen to be a consequence of variability in respiratory rates.

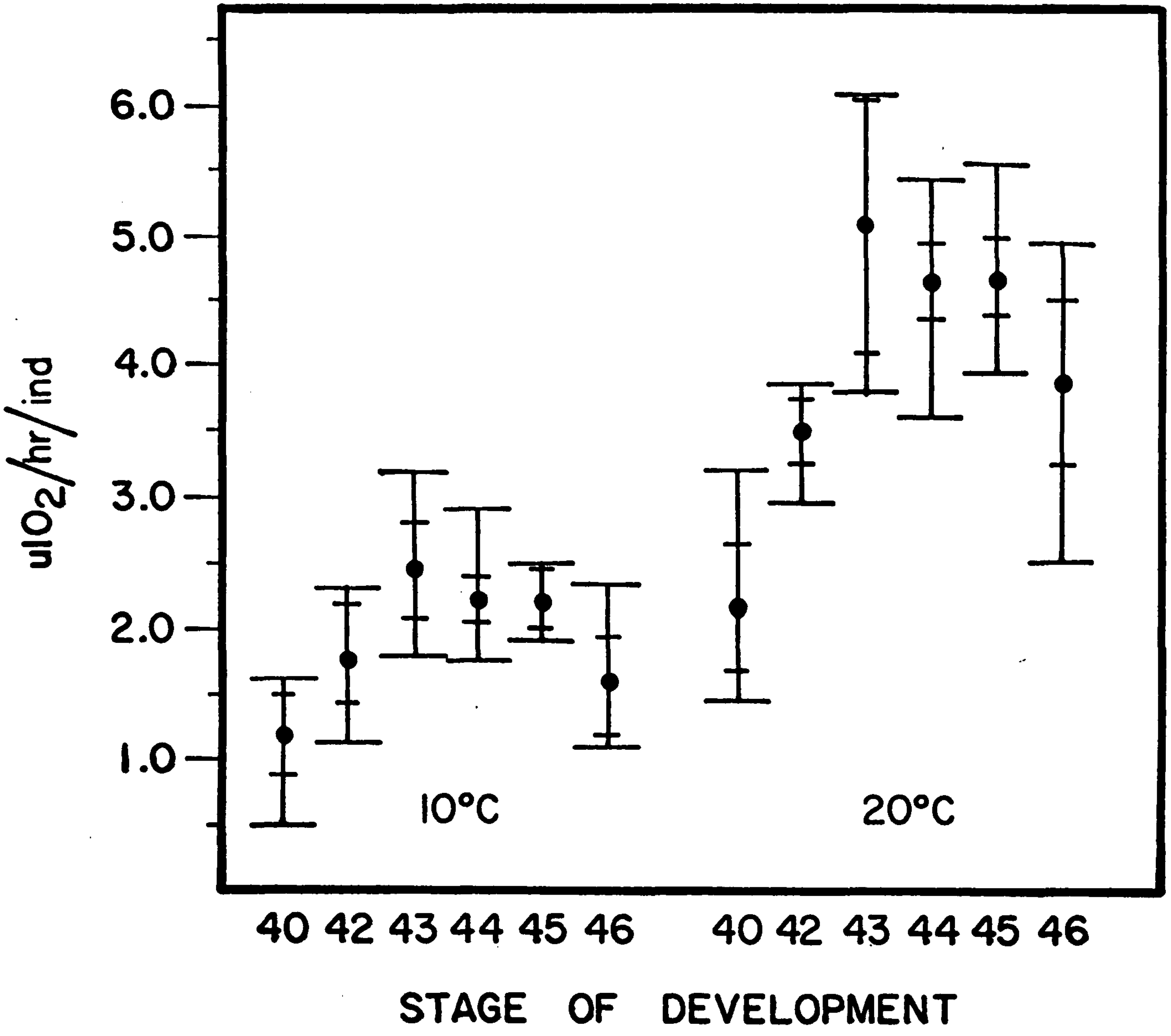
The discrepancy between the observed developmental times and those that are predicted by the above method are due to an underestimation of the average respiratory rate during the period when the larva is developing from a hatchling to a feedling. The respirometric data for feedlings indicate an increase in rate over the hatching stage. However, use of those rates would also underestimate the actual average respiratory rate. This is due to an increase followed by a decline in rate between the hatching and feeding stages (figure 28). Such an increase and decline was also reported by Wills (1936) for Taricha torosa and A. maculatum and by Hopkins and Handford (1943) for A. maculatum and A. tigrinum. The positive relationship between total length of the larvae and the rate of oxygen uptake is expected to be maintained throughout this developmental period, as it is in A. maculatum at the feeding stage where sample sizes are large ($r = 0.51$, $n = 18$, $p \lesssim 0.05$, at 10°C; $r = 0.52$, $n = 18$, $p \lesssim 0.05$, at 20°C). Therefore the true average rate, if it were known for larvae of individual females, would agree more exactly with the calorimetric data. This close agreement between these two independent techniques is taken to be an indication of the reliability of both.

Table 11. Developmental time between hatching and feeding as predicted from respiratory rates and calories utilized for *A. maculatum* and *A. tigrinum* larvae at two constant temperatures. (See text for further explanation.)

A. maculatum (10°C)

Total length at hatching (mm.)	Time between hatch and feed (observed h.)	calories used (observed)	O_2 consumed (ul/h.·ind.; observed)	Time between hatch and feed (predicted)
11.6	1180	6.58	0.513	2730
10.8	1253	5.74	0.306	3986
12.1	1222	6.84	0.642	2265
11.8	1198	6.83	0.564	2577
12.1	1179	7.12	0.642	2358
12.0	1175	6.98	0.616	2407
12.3	1270	7.13	0.693	2187
Correlation between hatchling size and predicted development time = -0.98				
A. maculatum (20°C) (legend as above)				
11.8	216	3.23	1.574	436
11.4	216	4.62	1.282	766
11.3	273	4.89	1.209	861
10.9	326	4.63	0.917	1074
10.8	296	4.51	0.844	1136
11.8	273	3.77	1.574	509
11.6	267	3.88	1.428	578
12.3	261	4.62	1.939	507
Correlation between hatchling size and predicted development time = -0.93				
A. tigrinum (10°C) (legend as above)				
14.7	551	7.57	1.534	1050
13.0	576	4.85	1.248	826
12.9	626	6.24	1.231	1078
11.5	1249	9.16	0.996	1957
11.5	920	7.55	0.996	1613
12.2	1084	7.06	1.114	1347
11.7	457	4.90	1.030	1012
Correlation between hatchling size and predicted development time = -0.59				
A. tigrinum (20°C) (legend as above)				
12.6	197	7.04	2.292	654
14.1	132	6.22	4.306	367
12.1	173	3.82	1.620	502
11.7	192	5.57	1.083	1094
Correlation between hatchling size and predicted development time = -0.80				

Figure 28. Means, ranges and standard deviations for ontogenetic changes in oxygen consumption rates for A. maculatum larvae at two constant temperatures. Harrison stage 40 = hatching; 46 = feeding.


6.0
5.0
4.0
3.0
2.0
1.0
ulO2/hr/ind
10°C
20°C
40 42 43 44 45 46
40 42 43 44 45 46
STAGE OF DEVELOPMENT

Feedling size

The effects of variability in ovum volume continue influencing larval development and result in feedlings with different total lengths at all temperatures (table 12). As with the effects of temperature on hatchling size, development at the colder temperatures (10°C and 10°⇄20° C) results in feedlings that are larger than those that developed at the warmer temperature of 20°C. The variability in ovum volume (which is closely related to calories contained per ovum; see above) is also positively related to the total calories contained in a feedling. In *A. tigrinum*, for example, the two-fold difference in calories contained per ovum is maintained at the feeding stage, where at 20°C, the feedlings of one female contain an average of 10 calories which those of another contain over 20 calories. Further examination of figure 25 indicates that at the feeding stage larvae that developed at colder temperatures used more energy than those that developed at the warmer temperatures (unlike development to the hatching stage where there was no indication of temperature effects on energy usage; see above). As previously mentioned, figure 25 also shows (by comparing slopes to the 100% efficiency relationship) that there is no consistent pattern between calories contained in the ovum and the number of calories used to become a feedling (as was the case in development to hatching). (In the case of temperature effects, the consistent pattern of elevation differences between regression lines is singled out while in the case of egg size effects, the general similarity of slopes to a value of 1.0 is emphasized.)

The efficiency of development

It might be possible for embryos and larvae that develop from ova that contain less energy to compensate for their apparent handicap by

Table 12. Feedling total lengths at 3 temperature regimes and the relationship between total length and ovum volume at those three temperatures for the larvae of individual females of four populations of *Ambystoma*. (* = $p \leq 0.05$; ** = $p \leq 0.01$)

	Feedling total length (mm.)			Correlation between ovum volume and feedling length		
population	10° C	10°⇄20° C	20° C	10° C	10°⇄20° C	20° C
A. opacum Ga.	18.3(SD = 0.76) (n = 4)	---------------	18.0(SD = 1.28) (n = 4)	0.541	-----	0.925
A. opacum N.J.	16.5(SD = 0.85) (n = 6)	16.3(SD = 0.99) (n = 6)	16.4(SD = 0.98) (n = 6)	0.892*	0.978**	0.866*
A. maculatum	15.5(SD = 0.70) (n = 15)	14.9(SD = 0.72) (n = 17)	14.8(SD = 0.74) (n = 16)	0.689**	0.792**	0.796**
A. tigrinum	18.0(SD = 1.06) (n = 16)	17.8(SD = 0.86) (n = 14)	17.5(SD = 1.19) (n = 13)	0.659**	0.517	0.593*

developing more efficiently. This could come about if, for example, morphogenesis is more energy-consuming for a large embryo. A major problem, however, exists in defining efficiency. Should larval length, or perhaps the energy they contain, be considered the more important measure? The two are not equivalent, as the data that have been presented show. Several measures of efficiency are presented here since a determination of the best measure would require extensive testing beyond the scope of this thesis. The first efficiency measure to be considered is how much of the initial energy contained in the egg winds up in the feedling. Table 13 illustrates that approximately 65% of the initial energy remains after development is complete but the variability is large (44.5 - 78.1%). There is a general tendency for ova with greater caloric contents to develop more efficiently. The relationship is borne out at $p \leq 0.05$ in 2 out of 11 possible correlations among the four populations (A. tigrinum at 10°C, $r = 0.79$, $n = 7$; A. maculatum at 20°C, $r = 0.81$, $n = 8$). Nevertheless, the remaining nine correlations are all positive. In addition, warmer temperatures increase efficiency as indicated by the mean efficiency values and the 2 out of 4 significant F-values (table 13).

The second efficiency measure to be considered is the length of larvae produced on a given amount of energy. The average Ambystoma feedling gets approximately 2.6 mm. of length out of every calorie metabolized. The large amount of variability in this value is not related to initial ovum energy in any of the 11 cases. It is, however, related to temperature (table 14). Even though feedlings that developed at colder temperatures are larger, they use many more calories to attain that size, resulting again in decreased efficiency at colder

Table 13. Mean efficiency values for the development of larvae of specific females as measured by the proportion of energy remaining in the feedlings relative to the energy contained in the ovum for development at 3 temperatures.

Population	Efficiency			Analysis of variance		
	10°C	10°-20° C	20°C	F	df	P
A. opacum$_{Ga.}$	0.60(SD = 0.020) (n = 5)	----------------	0.68(SD = 0.049) (n = 4)	12.6	1,7	0.01
A. opacum$_{N.J.}$	0.67(SD = 0.047) (n = 6)	0.73(SD = 0.054) (n = 6)	0.70(SD = 0.052) (n = 6)	2.2	2,15	NS
A. maculatum	0.50(SD = 0.016) (n = 6)	0.62(SD = 0.045) (n = 9)	0.67(SD = 0.061) (n = 8)	23.0	2,20	0.001
A. tigrinum	0.58(SD = 0.102) (n = 7)	0.67(SD = 0.064) (n = 4)	0.71(SD = 0.086) (n = 5)	3.0	2,13	NS

Table 14. Mean efficiency values for the development of larvae of specific females as measured by the length (mm.) the larvae attain on the calories utilized to the feeding stage for development at three temperatures.

Population		Efficiency		Analysis of variance		
	10°C	10°= 20° C	20°C	F	df	P
A. opacum$_{Ga.}$	2.04(SD = 0.135) (n = 5)	----------------	2.46(SD = 0.488) (n = 4)	3.4	1,7	NS
A. opacum$_{N.J.}$	2.94(SD = 0.315) (n = 6)	3.64(SD = 0.763) (n = 6)	3.26(SD = 0.576) (n = 6)	2.2	2,15	NS
A. maculatum	1.77(SD = 0.060) (n = 6)	2.26(SD = 0.278) (n = 9)	2.53(SD = 0.326) (n = 8)	14.3	2,20	0.001
A. tigrinum	2.26(SD = 0.316) (n = 7)	2.72(SD = 0.223) (n = 4)	3.01(SD = 0.511) (n = 5)	6.2	2,13	0.05

temperatures. This also becomes apparent if the increment in length between the hatching and feeding period is considered (Ryland and Nichols, 1967; Jones, 1972). *A. maculatum* and *A. opacum*-N.J. show significantly more growth per calorie between these two stages at 20°C than at 10°C (0.51 mm/calorie vs. 0.67 mm/calorie and 0.93 mm/calorie vs. 1.55 mm/calorie, respectively). *A. opacum*-Ga. also shows an increase in efficiency at the warmer temperature but due to small sample size the difference is not significant (0.84 vs. 0.97 mm/calorie). *A. tigrinum* efficiencies using this measure are approximately the same at the two temperatures (0.94 vs. 0.90 mm/calorie).

The feedlings that develop from more energy rich eggs should have more energy per unit length than feedlings that develop from smaller eggs, since an embryo that starts off with a greater amount of energy and uses that energy more efficiently in terms of energy content of the feedling does not get more length out of every calorie metabolized. Out of the 11 correlations that can be run between calories contained per ovum and calories per mm. feedling, 8 are significant at the 0.05 level. At warmer temperatures the feedling contains more energy per unit length than at colder temperatures ($P \leq 0.005$ in 2 our of 4 cases and not significant in the other 2 although the variability is in the proper direction).

In summary, the analysis of developmental efficiencies presented here indicates that feedlings that result from eggs with greater caloric content develop more efficiently, and, furthermore, development at warmer temperatures is more efficient. The alternating environment does not decrease efficiency, but as before, values under this temperature regime are intermediate and generally closer to values obtained at the constant 20°C temperature.

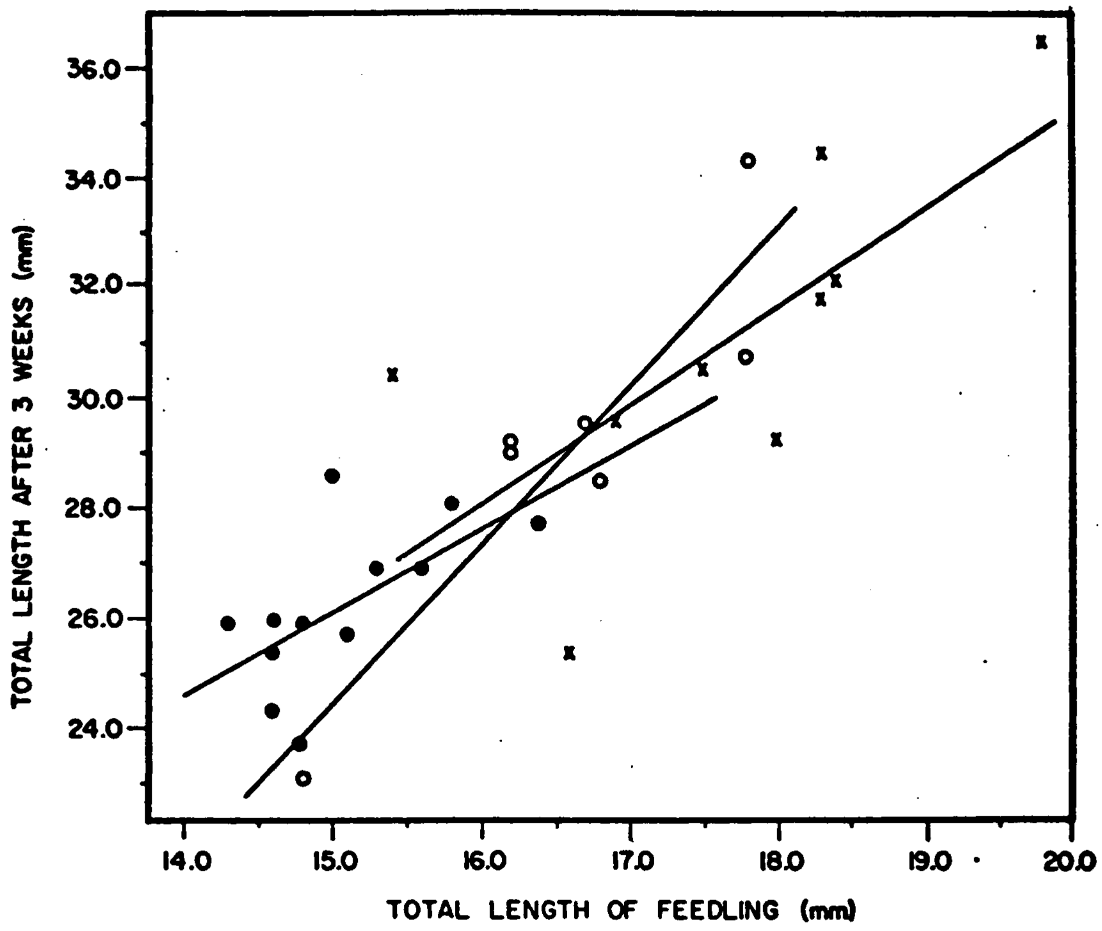
Post-feeding early larval growth

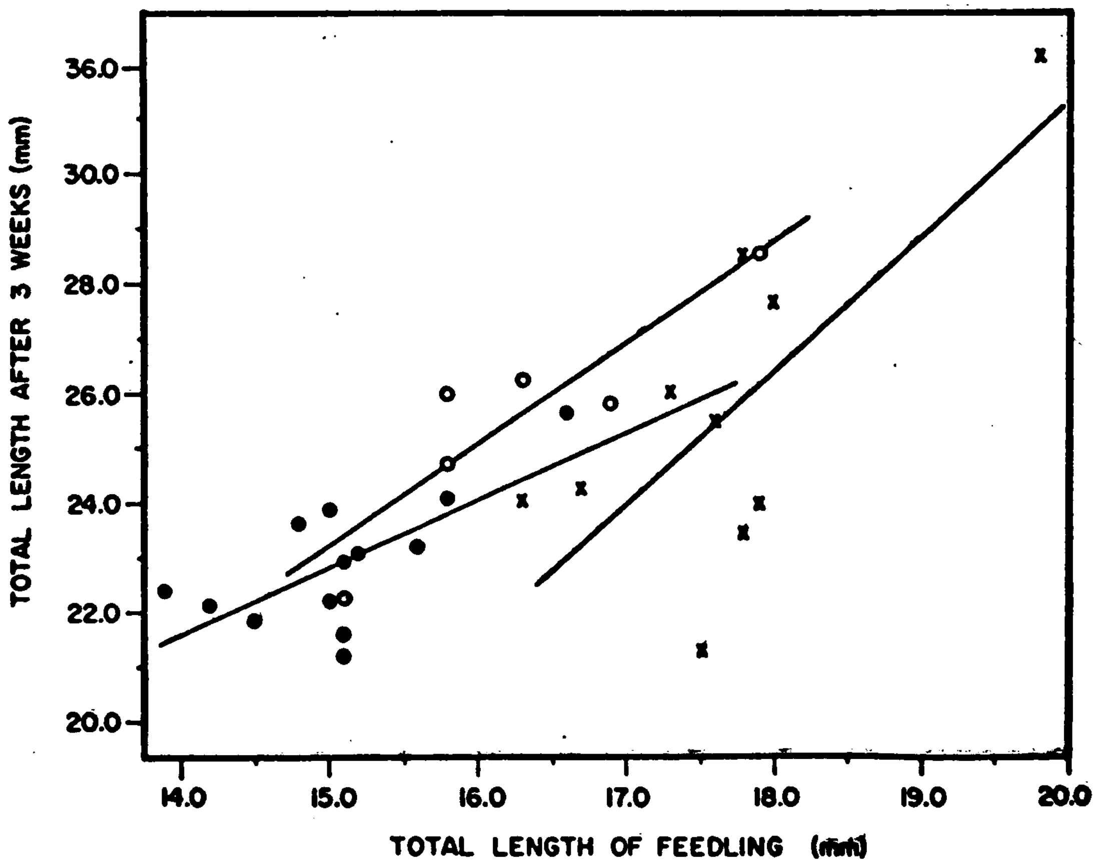
As has already been shown, larvae that differ in total length differ in energy demand, as indicated by the relationship between hatchling size and oxygen consuption rate (see figure 26 and 27). While this increased demand must partly be due to increased maintenance requirements, it also reflects a greater potential for the mobilization of energy which can be used for growth. To test this prediction larvae were fed for a period of three weeks (see "Materials and Methods") and the relationship between feedling total length and larval total length after three weeks of growth was determined. No data were obtained for *A. opacum*-Ga. due to lack of sufficient sample, and *A. maculatum* does not feed at 10°C. As table 15 indicates, out of the remaining 8 possible correlations all are significant at the 0.05 level, meaning that larger feedlings do give rise to larger larvae at least during the period of early larval growth under conditions of unlimited food supply. The relationships for 20°C and 10°⇌20°C are plotted in figure 29. All slopes are greater than 1.0, even though only 2 out of 8 are significantly greater than 1.0 at a level of significance of 5.0%. That the slopes are in fact greater than 1.0 is made even more certain if it is recalled that least squares regression tends to underestimate slopes when correlations depart from 1.0. This means that as feedling size increases growth rates tend to increase as well. This explains for example, the higher growth rates of *A. tigrinum* and the larger *A. opacum* larvae relative to *A. maculatum*. In fact, figure 29 tends to suggest that the phenomenon may be a characteristic of at least these members of the genus and many of the differences attributed to species could be

Table 15. Relationship between total length after three weeks of growth (Y) to the total length of the feedling (X) at three different temperatures for three populations of Ambystoma. (r = product moment correlation; * = $p \leq 0.05$, ** = $p \leq 0.01$).

population	temperature	N	slope	intercept	r	deviation of slope from 1.0 P
A. opacum N.J.	10°C	4	1.55	- 5.86	0.97*	NS
	10°= 20° C	6	1.88	- 5.01	0.90*	NS
	20°C	7	2.91	-19.14	0.91**	0.01
A. maculatum	10° C	-	----	------	----	----
	10°= 20° C	13	1.22	4.55	0.69**	NS
	20°C	12	1.50	3.59	0.62*	NS
A. tigrinum	10°C	12	1.77	-12.77	0.86**	0.05
	10°= 20° C	10	2.43	-17.17	0.72*	NS
	20°C	9	1.80	- 0.67	0.72*	NS

Figure 29. Length of larvae after three weeks of growth plotted against the length of the feedling. a = development at 20°C; b = development at 10°=20°C. open circles = *A. opacum*$_{\text{N.J.}}$; closed circles = *A. maculatum*; crosses = *A. tigrinum*.

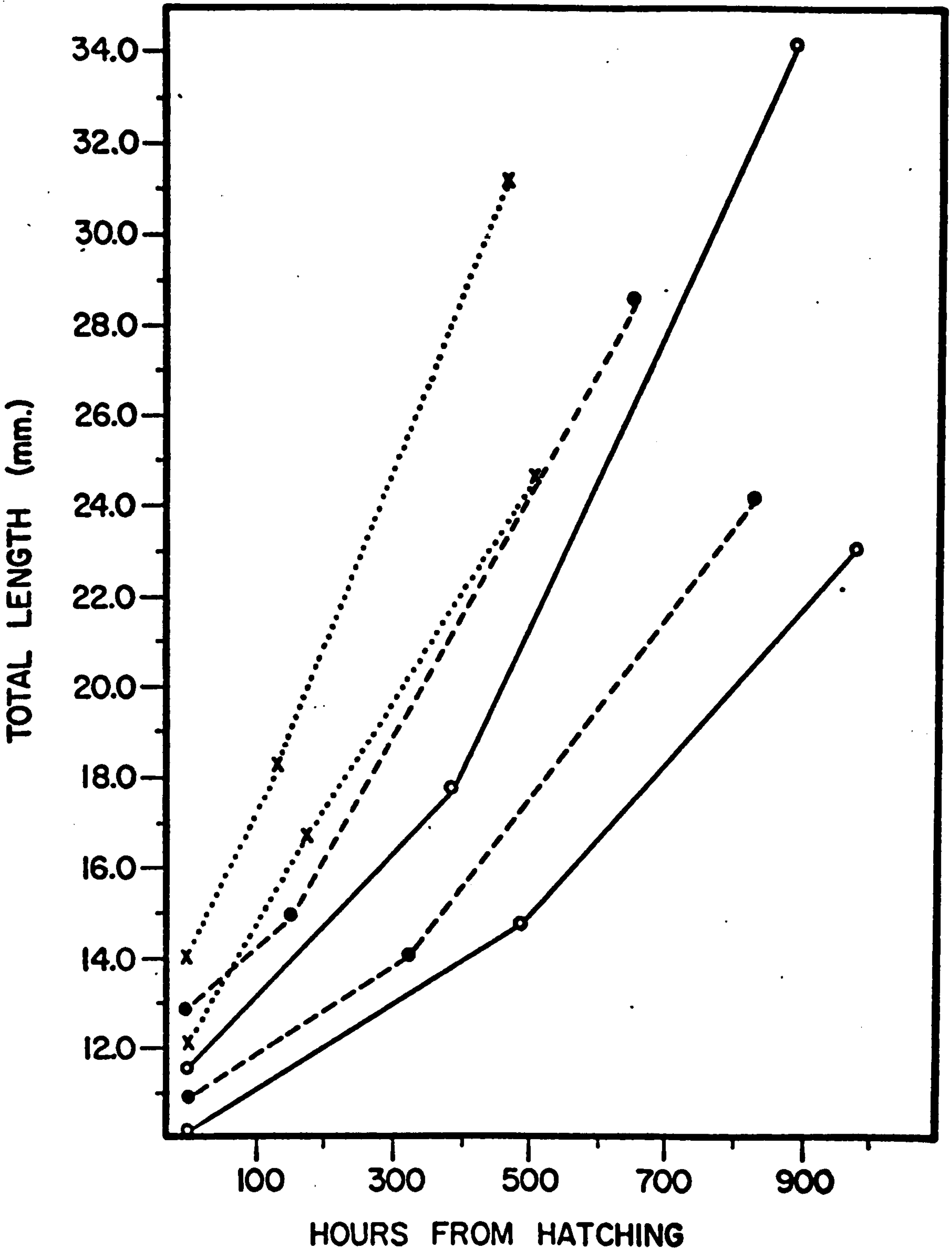

TOTAL LENGTH AFTER 3 WEEKS (mm)
24.0
26.0
28.0
30.0
32.0
34.0
36.0
14.0
15.0
16.0
17.0
18.0
19.0
20.0
TOTAL LENGTH OF FEEDLING (mm)


TOTAL LENGTH AFTER 3 WEEKS (mm)
20.0
22.0
24.0
26.0
28.0
30.0
36.0
14.0
15.0
16.0
17.0
18.0
19.0
20.0
TOTAL LENGTH OF FEEDLING (mm)

at least partially explained on the basis of larval size differences.

As a partial summary of the data presented, the growth patterns at 20°C for the larvae of two different females from each of the species are shown in figure 30. The larvae of specific females were selected so that the larvae with the most extreme values for each population would be presented in order to make the relationship (which has already been established; see above) most clear. The two females from each species produced eggs of different sizes. This resulted in hatchlings of different sizes at time zero. As has been shown, larger hatchlings give rise to larger feedlings, but the larger hatchlings, in the case of A. opacum-N.J., for example, begin feeding four days earlier than the smaller hatchlings. In the case of A. maculatum seven days separates the onset of feeding and in the case of A. tigrinum one and one-half days. Returning to A. opacum-N.J., it is seen that feedlings differ by approximately 3.0mm. of total length. However, by the time the smaller hatchlings begin to feed the larger ones have already grown to be approximately 6.5 mm. larger. In A. maculatum the difference is even more extreme. Size-related differences in growth rate would increase the difference in larval size throughout this period of growth. Similar relationships obtain at the colder temperatures as shown by the data presented previously.

Figure 30. Total length of larvae plotted against hours since attainment of the hatching stage. (The second point along each curve represents the feeding stage.) (Symbols as in figure 29; see text for further explanation.)


34.0
32.0
30.0
28.0
26.0
24.0
22.0
20.0
18.0
16.0
14.0
12.0
TOTAL LENGTH (mm.)
100
300
500
700
900
HOURS FROM HATCHING

DISCUSSION

There are as many reproductive strategies as there are kinds of organisms. This is not surprising since a large variety of traits (e.g., age at first reproduction, age-specific reproductive effort) make up a reproductive strategy and each of them varies considerably across taxa. The precise description of a particular species' strategy is not particularly useful in helping to explain why a species has evolved that strategy. The problem is not unlike that of determining a species' niche. It includes the subproblems of discerning the relevant abiotic and biotic components of the environment with which a strategy must be in harmony. There is the additional problem of measuring the relevant aspects of the phenotype while separating the effects of genetic and environmentally induced variation. With the possibility of multiple optimal adaptive solutions in specific environments (Schaffer and Rosenzweig, 1977) and of highly variable environmental parameters through time in specific localities, the understanding of life histories is a formidable problem and has only begun to be attempted in particular cases, with empiricists trying to keep up with a burgeoning theory (see Stearns, 1976 and 1977 for a review).

Any part of the zygote-to-zygote life cycle in a sexually reproducing species is fair game in a study of reproductive adaptations. Here I choose to consider the link between the total reproductive mass that a female produces at a specific time and the subsequent effects of partitioning that mass into portions of varying size. In the first instance, I consider the effects of body size as a design constraint which strongly influences the sorts of reproductive traits that can evolve, which in turn can determine a large part of the remaining strategy. The trophic position of an organism (Wilbur _et al._, 1974) and its phylogenetic history (Maiorana, 1967a) can also impose constraints on life-history adaptations. Secondly, I consider how a single constraint is not necessarily confined to a single generation but exists between generations in the sense that developmental constraints on offspring will influence the partitioning pattern of the reproductive mass. By seeking the major constraints on an organism's reproductive traits it should become easier to explain the diversity that exists in reproductive strategies.

RATIONALE

It was suggested by Savage (1952) that the size of amphibian populations may be effectively regulated during the premetamorphic phase of the life cycle (excluding direct developing forms). Since that time numerous articles have appeared which have shown premetamorphic mortality to be substantial, imposing a strong filter on the numbers and kinds of individuals that are recruited into the adult population. The mortality factors that have been shown or suggested to be important are numerous and include such variables as temperature, ephemeral habitats, rainfall, disease, predation, competition and chemical effects (Martof, 1956; Turner, 1960 and 1962; Herreid and Kinney, 1966; Worthington, 1968; Brockelman, 1969; Anderson _et al._, 1971; Wilbur 1971 and 1972; Calef, 1973; DeBenedictis, 1974; Shoop, 1974; Collins, 1975; Heyer _et al._, 1975; Heyer, 1976; Pough, 1976; Rose and Armentrout, 1976; Woodruff, 1976). In all of these studies embryos or larvae within a population are treated as a homogeneous group with potential mortality factors acting with equal probability on different individuals (but see Savage, 1952). Such an approach does not take into account potential differences that exist among the offspring of specific females within the population and, therefore, while providing general information about population regulation, provide limited insight into the ways in which natural selection can bring about adaptive changes within a population.

Ovum size is the characteristic singled out in this study that can be used to discriminate among individuals subjected to embryonic and larval mortality factors. The sizes of eggs presumably affect the survival of offspring in various ways and also determine how many of them a parent can produce (see below). Interspecific variability in egg size among amphibians is well known. It is a character that herpetologists have always felt worthwhile measuring. It was possible to construct the interspecific relationships between body volume, egg volume and reproductive mode presented in figure 8 (modified from Salthe, 1969) because of the efforts of many investigators. Some authors felt it worthwhile to look at interpopulational variability in ovum size (Slater, 1937; Twitty, 1942; DuShane and Hutchinson, 1944; Moore, 1949; Volpe et al., 1961; Pettus and Angleton, 1967; Nussbaum and Tait, 1977). Others have found variation within populations (McAlister, 1962; Hönig, 1966; Collins, 1975). No one has dealt specifically with the magnitude of this intrapopulational variability or its ramifications either on development or on the reproductive potential of the female. With reference to the latter, variation in clutch volume among females within populations has been calculated by multiplying a mean egg volume for the population by egg number per female (Tilley, 1968 and Houck, 1977a and 1977b). Bruce (1969) considers egg size to be a constant among three species of primitive plethodontids. By using mean egg sizes, differences in reproductive output are ascribed to clutch size differences alone. Wilbur and Collins (1973) explicitly considered variability in offspring size to be unimportant in their model predicting the timing of amphibian metamorphosis.

As the data presented here (figure 2) indicate, there is a large and significant amount of female-specific variability in egg size within five populations of Ambystoma. As has been shown and will be discussed (see below), this variability affects the clutch sizes and the clutch volumes of the females and fundamental aspects of the developmental processes of their offspring.

The remainder of the discussion is divided into two main sections. The first considers the implications of variability in ovum size on the relationship between female body size, clutch size and clutch volume. The second considers the effects of this same variability on development.

THE ALLOMETRY OF REPRODUCTION

In this section I wish to show how body size can be used in analyzing reproductive patterns, where often the only data available are adult body size, ovum size, and egg number. The importance of body size in determining constraints on adaptations has recently been summarized (Pilbeam and Gould, 1974; Gould, 1975a and 1975b), but the implications of such constraints for reproductive biology have as yet received little attention. For example, if life-history considerations lead us to predict that one species will have a larger clutch size than another, we may specify more precisely what "larger" means by using an empirically determined scaling model. In some cases it may mean an absolutely smaller clutch size, but one larger than a value extrapolated from an allometric relationship. In short, knowledge of scaling generalizations will allow us to pose more precise null hypotheses against which sample observations can be tested (Gould, 1975a and 1975b).

Interspecific allometric relationships between body size and various reproductive parameters have been described only in a few cases, beginning with Huxley's (1927) study of the relationship between egg weight and body weight in birds. Such relationships were also described between body length and egg number in spiders (Peterson, 1950) and prawns (Kurtén, 1953). Only more recently has there been any attempt to use allometric relationships for specific purposes. For example, Leutenegger (1972) employed the relationship between fetal weight and maternal weight in primates as a basis for statements concerning possible past selection pressures on head size in relation to birth difficulty in the lineage leading to man. In addition, extending the allometric approach to the intraspecific level can in some cases provide an alternative to food limitation theories (e.g., Lack, 1968) for the regulation of clutch size. This approach was begun by Hubbs et al. (1968) with reference to a teleost fish. They considered body cavity volume limitation along with energetic constraints imposed by the surface area of the gut as contenders for the major share in explaining clutch size.

In this study, null hypotheses concerning variation in total clutch volume, clutch size and ovum size are formulated and used as models to be tested in populations of Ambystoma. The null hypotheses consist of static "mouse-to-elephant" interspecific relationships (of the form $\log y = a \log x + \log b$) and are used to make predictions of the means of each sample population. Statistically significant deviation of sample means ($p \leq 0.05$) may suggest interesting ecological specializations. An alternative test of whether the slope generated by the interspecific data could reasonably describe a line through the population data was not considered because there is no reason to suppose that intra- and interspecific allometric relationships would share the same slope. The latter condition seems unlikely because sources of explained variability are not the same in the two cases despite the fact that size is important in both. Thus, within a population the major source of variability will come from the reproductive adaptations of individuals interacting with age structure and environmental variation, while between species it comes largely from accumulated genetic differences.

Justification of the approach

Two frequently accessible components of any reproductive strategy are the number and size of the eggs or offspring that are produced. It is well known that these components often vary in ways that are related to the size of the parental organism at interspecific, interpopulational and intrapopulational levels. Combined, these two components represent the total reproductive mass produced during any given breeding period. In most salamanders this entire mass is present in the body of the parent at one time. As body size changes, parts of an organism such as bone, pancreas, or mature ovary may also change allowing for the maintenance of the individual as an adaptively integrated unit. In the case of the former two organs it is clear that body size increases will require concommitant increases for the organism to continue functioning. With respect to mature ovaries, however, there is no a priori reason to presume that larger animals require larger ones to insure the continued survival of their genes. On the other hand, parental body size can place an upper limit upon the size of an ovary that can be adequately maintained.

The empirical literature on practically every animal group generally shows positive associations between reproductive output and body size regardless of the level of organization that is being considered, be it inter- or intraspecific. In analyzing data of this sort geometric similarity rather than allometry of functional scaling has been assumed to be the rule. Failure to distinguish between these two alternatives results in faulty conclusions since the data are being tested against erroneous hypotheses. This is one of the reasons that should have led to the recent abandonment of the concept of reproductive effort as being approximated by a ratio of parental mass to offspring mass (see an attack on this concept on other grounds by Hirshfield and Tinkle, 1975). Such ratios, which change with size due to the allometric nature of the component variables, can provide useful as well as readily accessible information about the reproductive biology of particular populations provided that the allometric nature of the data being used has previously been established. With this viewpoint in mind, reproductive data from many salamander species have been amassed in order to interpret similar data about specific populations.

The interspecific allometric relationship

The interspecific relationship presented in figure 9 indicates that a large portion (81%) of the variability in clutch volume is explained by variability in body size. This relationship signifies that there is a constraint put upon total clutch volume during any breeding season by the size of a salamander's body. Since there is no reason to suppose that every salamander species is maximizing its potential output during every breeding season (and there is evidence to the contrary in Maiorana, 1976b; and see below), the elevation of the line in figure 9 represents a submaximal relationship for salamanders in general. A particular sample mean may lie above the line, indicating that clutch volume of the population tends in some degree to approach the maximum allowed by size constraints. The more closely a population approaches its maximum, the more likely it is to find a trade-off between the number and size of offspring produced. This is corroborated by the data on ambystomatid salamanders in which A. tigrinum, with an unusually large total clutch volume (figure 12), is the only population in which there is a significant negative partial correlation between egg size and clutch size for a female of a specific body size ($r_{partial} = -0.658$; $n = 15$; $p \leq 0.05$). Similarly, the further a population is from the maximum, the more the variation in reproductive output may be correlated with such factors as energy available for reproduction.

The generality of the clutch volume - body volume model for salamanders is indicated by the robustness of the relationship when examined for interspecific subsets (different reproductive modes). There is no change in either slope or elevation. The slope of the relationship ($a = 0.640$) indicates that geometric similarity is not being maintained. Therefore, all things being equal, one should expect a large salamander to have less of its body volume occupied by its clutch than a smaller one; the relationship is negatively allometric.

Comparison of interspecific and intraspecific allometric relationships

Within some restricted adaptive zone an interspecific slope describes the dependence of a parameter on body size. When a lineage has not evolved away from a particular adaptive zone the relationship can be used to predict the mean value of a trait for a species of a particular size (Gould, 1975a and 1975b). The relationship does not impose a strong interspecific constraint since a lineage may evolve from one adaptive zone to another. An intrapopulational slope, however, has a considerably different meaning; it reflects the operative genetic constraints associated with a trait interacting with proximate environmental factors. Individuals living in an average environment (be it a fluctuating one or not) cannot violate these constraints by much and still compete successfully.

A number of factors may contribute to the dissimilarity that is generally found between inter- and intraspecific body size dependent relationships (e.g., table 2). Within a population, body size itself is limited relative to the entire range of body size found between species - it is a truncated relationship. Furthermore, among organisms which exhibit indeterminate growth (as do salamanders), an individual can attain all possible sizes sequentially, and so the intrapopulational relationship contains a good deal of ontogenetic variation. This contributes to the lack of similarity between the two kinds of slope because in the interspecific case only organisms of an average size for each species are compared. Phylogeny does not parallel ontogeny in these cases. (See Kaplan and Salthe, in press, for an extension of this discussion.)

In specific cases, examination of the values of the slopes of inter- and intraspecific relationships via dimensional analysis may provide insight into their meaning (Guenther, 1975). The interspecific relationship between clutch volume and body volume can be evaluated by means of several different scaling criteria, given that the independent variable is a volume. If a simple "mechanical" criterion is selected, examination of Guenther's tables suggests that the allometric exponent ($a = 0.640$, which is close to 0.66; table 2) may reasonably be interpreted as being imposed by changes in distensibility of the body wall with changing size. The animals may then be viewed as packing into the available space an amount of yolk that approximates the maximal amount possible. The same explanation could be used in connection with the slope of 0.67 found by Rahn _et_ _al_. (1975) for the relationship between egg weights and body weights among species of birds. In birds only a single egg is manufactured and processed at a time (although others may be in less mature stages of development in the ovary), and this egg must be accommodated by a distensible reproductive tract. Within these populations of ambystomatids (table 2), total clutch volume appears to scale on body volume with an exponent of 1.00, not 0.66. If the "functions of organs and their association into systems" (Guenther, 1975) is considered to be a more appropriate criterion for interpretation of intrapopulational data, then a "biological" as opposed to a "mechanical" scaling criterion should be applied, in which case distensibility would scale on body volume with an exponent of 1.00.

While it is felt that distensibility is the simplest explanation for the observed slopes, views that energy availability may be the more significant factor cannot yet be discounted. Hubbs *et al.* (1968) explained clutch size variability in a teleost fish as being limited by the surface area of the gut (see also Leutenegger, 1976). While this explanation is consistent with the interspecific data presented here, slopes of 1.00 in the intrapopulational data are inconsistent with their view. However, under the "biological" scaling criterion of Guenther, the amount of energy used through a given organ to accomplish some task does scale with an exponent of 1.00. Therefore, while potentially useful, the dimensional analysis approach has not allowed discrimination between these major contending factors.

Applications to reproductive patterns

A reproductive strategy involves more than the total clutch volume produced by an individual during any given breeding season. The relative size of a mature egg mass that is maintained within the body of a female salamander is, however, presumably influenced by natural selection. As has already been noted, the size of the female constrains the size of the egg mass. However, within one body size range a variety of egg mass sizes is possible. Selection results in a compromise which puts as little strain as possible on the female (detracts as little as possible from her future ability to reproduce) and yet produces a sufficient egg mass to make the breeding effort worthwhile. Long term environmental differences appearing in the form of the differential survival either of the females themselves or of their offspring will play a significant role in determining the size of the egg mass during any breeding season (assuming energy availability to be minimally varying). Other factors being equal, relatively small egg masses will be associated with increased iteroparity and longevity while larger egg masses will be associated with decreased iteroparity and shorter lifespans. Environmental conditions favoring either of these strategies will vary, and a large theoretical literature exists that predicts which strategy will evolve in particular situations (see Pianka, 1976 and Stearns, 1976 for reviews). Once a particular strategy has been set ultimately by selection, the size of a particular egg mass can be affected proximately by differences, for example, in energy or time available for yolking eggs.

The usefulness of the clutch volume - body volume modeling approach is made most obvious by comparing the *A. tigrinum* population with that of *A. opacum*-N.J. (figure 14). Both have essentially the same proportion of their body volume occupied by a clutch (13.6%). Without the allometric model, an hypothesis of geometric similarity would be assumed and the two populations would be considered to be exhibiting a similar adaptation. With the model, however, it is seen that they are in fact quite different. *A. tigrinum* has a clutch mass 1.9 times larger than expected, indicating that it is more closely approximating its upper limit to clutch volume than is the *A. opacum* population. Only the *A. tigrinum* populations shows a trade-off between egg size and egg number, indicating that its egg mass is indeed being constrained by body size. When a population is not approaching its maximum clutch volume, more of the variability in that volume should be explainable by factors such as energy availability among individuals during the yolking period (Ballinger, 1977; Houck, 1977b) and perhaps even by variability in tail size due to predation (Maiorana, 1977). Similarly, seasonal variations in the mean clutch volume of a population may also be explained by different feeding opportunities during different seasons (Nichols *et al.*, 1976), and again these differences would be most apparent in a population not near its maximum clutch volume, for example the *A. maculatum* population, which is producing a clutch on the average only 69% as large as expected.

Presumably it will be easiest to determine causes of deviations from the model in the most extreme cases, (which in this study are *A. tigrinum* and *A. maculatum*). Unfortunately, much of the relevant data required, such as age at first reproduction, breeding frequency, and longevity are poorly known and are themselves probably subject to environmentally-induced variation. It is known for example that *A. tigrinum* has a rapid growth rate (Bishop, 1941) and possibly a short lifespan (Wilbur, 1977). These characters are concordant with the unusually large clutch volume of this population. *A. maculatum* has been observed skipping breeding seasons (Husting, 1965) which is consistent with its low clutch volume and presumably longer lifespan. Of greater significance, however, is the extremely high embryonic mortality that is an apparently characteristic feature of this population of *A. tigrinum*, with 80% of the embryos not reaching the hatching stage and 97% not surviving metamorphosis (Anderson *et al.*, 1971; pers. obs.). Here one might expect, based on theoretical considerations, that total clutch mass would be reduced and the number of breeding periods increased throughout the life of the adult (Charnov and Schaffer, 1973; Schaffer, 1974). If, however, this high embryonic mortality is a consistent (rather than variable) feature of this particular population (as it seems to be), then higher birth rates during any season could be an alternate way of insuring genetic survival (Brockelman, 1975), and this indeed seems to be the option taken through evolutionary time by individuals in this population. Interpopulational comparisons would be especially useful in this particular situation to determine how regularly *A. tigrinum* populations produce extremely large clutch masses for their size. Similarly, if the reproductive strategy of *A. tigrinum* had evolved in another kind of environment, then the evolution of a large clutch mass in response to increased embryonic mortality may have been the only readily accessible change in strategy allowed by its preadaptations. While interspecific comparisons are not particularly useful to substantiate such a point of view, it is at least thought-provoking to note that the study population of A. maculatum, with a much reduced clutch volume, suffered virtually no prehatching mortality during the course of this study (pers. obs.), while a population of A. maculatum in Massachusetts which suffered higher mortality rates had size-specific clutches that were nearly double those of the population reported here (Shoop, 1974 and pers. comm.). It seems, then, that an overall reproductive strategy can be interpreted according to theoretical predictions but that interpopulational variation in local mortality schedules may affect the size of the clutch mass once the overall strategy has been fixed. With conservative ovum sizes apparently imposed on Ambystoma (this work - see below) this is tantamount to changing the numbers of offspring. This method of analysis will allow recognition of the average reproductive strategy for a species (with respect to clutch mass) once sufficient interpopulational data have been accumulated. Deviations (or their lack) of particular populations from the average species' value may suggest possible environmental causes and their recency.

The interspecific models that are presented for clutch size and ovum volume for mode I salamanders are not as useful as the clutch volume model describing all species of salamanders. This is due to the lower correlation values for the former two models (tables 3 and 4) and the subsequent uncertainty in the slopes of the regression lines. This is expected since only total clutch volume can be constrained by body size. A comparison of the clutch size and ovum size models with the clutch volume model makes it evident that the variability in clutch volume among populations (discussed above) is most readily explained by the variability in clutch size. In reference to the relationship for pond breeders (figure 13), the population of *A. tigrinum* can be seen to have a larger clutch volume than expected as a result of the production of a large number of eggs (D.Q. = 2.62; table 3), while *A. maculatum* with its relatively small clutch volume is producing relatively few (D.Q. = 0.69). *A. opacum*-S.C., because of its combination of a "normal" clutch volume with relatively large ova is producing fewer eggs than expected on the basis of the model. In reference to *A. opacum* in general, this analysis clearly indicates that its change in mode of egg deposition has had no effect on either clutch volume, clutch size, or egg size, which have all remained quite typical for an *Ambystoma* of its particular size (see also Kaplan and Crump, 1978). Since neither egg number nor size is constrained by body size, an analysis of slopes in these cases is inappropriate. As data for these variables accumulate, however, these models will become increasingly useful. The relative position of each species compared to other pond breeding salamanders will become more clear and the causes of interpopulational variability will become analyzable.

THE ENERGETICS OF DEVELOPMENT

What is the significance of the intrapopulational variability in ovum size on the developing offspring?

Energy content of eggs

In a progression from oligolecithal to telolecithal eggs there is an increase in the proportion of the egg that is composed of lipids (see review in Follet and Redshaw, 1974). This would be reflected in increases in weight-specific energy contents. Even among species which produce strictly telolecithal eggs, there is significant interspecific variation in lipid, protein, carbohydrate and weight-specific energy content, as revealed for example, in the avian literature (see Ricklefs, 1974, for review). This interspecific variability among birds has been related to differences in altricial and precocial development. Tinkle and Hadley (1973 and 1975) showed that there are significant differences in the ash-free dry weight-specific caloric content among the eggs of 10 species of lizards (values range from 5.87 to 7.20 cal/mg AFDW) and they noted that a constant value across species cannot be assumed as previously suggested by Ballinger and Clark (1973).

Among the amphibians, Slobodkin (1962) was the first to report weight-specific caloric values corrected for ash and found that *Ambystoma maculatum* and *Rana pipiens* ova are identical at a value of 6.0 cal/mg AFDW. Boyd and Goodyear (1971) reported that there are differences in the percentage of protein and fat content among 15 amphibian species. [They fail however to provide statistical validity to their report and make the assumption that amphibian eggs are devoid of carbohydrate, which is puzzling in light of the literature being replete with references to carbohydrate metabolism during early amphibian embryogenesis (see, for example, Barth and Barth, 1954 and Løvtrup, 1959)]. Similar interspecific differences in fat, protein, and carbohydrate content of eggs have been reported previously (see Salthe, 1969, for references). Fitzpatrick (1973) reports that mature ovaries of the salamander *Desmognathus ochrophaeus* contain 6.460 cal/mg

AFDW while Bell (1977) reports ripe ovaries of *Triturus vulgaris* to contain a surprisingly low value of 5.72 cal/mg AFDW. Crump and Kaplan (in prep.) found significant interspecific differences in weight-specific energy content of ova among several species of tropical tree frogs of the genus *Hyla*, with values ranging from 6.04 to 6.43 cal/mg AFDW.

In this study significant differences both within and between species of *Ambystoma* have been found with mean values for populations ranging from 6.39 to 6.65 cal/mg AFDW. It can be concluded that yolky vertebrate eggs contain a good deal of lipid accounting for their relatively high energy content on a weight-specific basis. A constant energy value cannot be assumed, and needs to be determined in each case if accurate values are required. If only approximations are needed, however, it should be noted with reference to amphibian eggs in general, that the amount of material (dry weight) contained in a specific volume of egg is strikingly similar in the *Hyla* study by Crump and Kaplan cited above and in this study (0.45 to 0.50 mgDW/mm^3 in both cases). This should prove useful in thumbnail approximations of dry weight and energy content in amphibian eggs.

Variability in various energy parameters (cal/mm^3, mg/mm^3 and cal/mg) and in the total energy content of ova within populations has (to my knowledge) never before been determined for any animal group and as the data presented here for weight-specific caloric content (figure 15) and total energy content of ova (figure 16) show, the variability is considerable and closely related to ovum size (figure 17). Before the ramifications of this variability is assessed with reference to the fitness of the parent, the implications of the variability for the offspring need to be determined. [If starting life with twice as much energy as another embryo is of no advantage within a population, then the added cost to the parent either in actual energy required for vitellogenesis or in reduced egg numbers (see above) certainly cannot be worth it.] The remainder of this discussion is devoted to this topic.

Larval size and egg size

In anamniotes, it is generally conceded that the larger an egg is the larger the resultant offspring will be upon hatching. Up to now, there have been no data showing intrapopulational correlations between these variables. Salthe (1969) and Salthe and Duellman (1973) noted this effect in interspecific relationships among salamanders and frogs respectively. Svärdson (1949) and Marshall (1953) found similar relationships among fish species. At the interpopulational level, Blaxter and Hempel (1963) found that populations of herring that produced larger eggs produced larger larvae at the hatching and feeding stages. Rosenthal (1969) showed that herring larvae from populations with larger eggs were more capable of trapping food. DuShane and Hutchinson (1944) found that at the time of first feeding, a population of *Ambystoma maculatum* larvae that developed from larger eggs were larger than those of a population that developed from smaller eggs. Similarly, Nussbaum and Tait (1977) report that in *Rhyacotriton olympicus*, a population that produced larger ova also had larger hatchlings and larger metamorphs. At the intrapopulational level, Bell (1977) found that ova from larger females (perhaps implying larger ova) developed into larger larvae in the smooth newt, *Triturus vulgaris*.

As has been shown here (see results and figures 21, 23 and 25 and table 13), within populations different investment in individual eggs by different females can result in highly significant effects on total length and energy content of both hatchling and feedling. If this increase in size, however, is obtained at a cost of increased developmental time or at a great energetic expense relative to the initial amount of energy that development begins with, then the presumed benefit to the fitness of the female parent by producing larger larvae may be negated (see Bagenal, 1969). As is discussed below, these two effects (size-related differences in developmental time and efficiency of development) do not negate each other but in actuality enhance the probable advantage associated with larger initial investments in specific offspring.

Developmental rate and egg size

Using interspecific data, it has been emphasized repeatedly that the larger an anuran egg is the more slowly it develops (McLaren, 1965; McLaren and Cooley, 1972; Salthe and Duellman, 1973; Kuramoto, 1975). This relationship supposedly imposes limitations on variability in egg size to conform to the requirements of developmental rate. Difficulty in comparing interspecific developmental rates, due to environmental temperature and genetic differences, makes this relationship quite ambiguous. Moreover, the relationship does not hold in all interspecific cases (see for example, Crump, 1974).

The rate at which an embryo develops is an important ecological characteristic. Fast rates of development may allow for increased survivorship in temporary ponds. They may also allow for more rapid growth in competitive and predator-rich environments in which size may be important for an individual's survival. Fast development (to the age of first reproduction) can add greatly to the reproductive potential of individuals (Lewontin, 1965). Yet slow developmental rates may be advantageous in some environments, if for example, lengthened pre-hatching periods reduce predation on mobile (more exposed) larvae. Even within a single habitat fast rates may be advantageous at some times and slow rates advantageous at others (Shoop, 1974). No generalizations concerning the adaptive values of developmental rates can be made. This tends to weaken one's faith in using interspecific data to construct causal relationships between developmental rate and egg size.

An analogous situation has emerged in the invertebrate literature. Vance's models (1973a and 1973b), that predict the evolution of differing reproductive modes among marine benthic invertebrates, assume a positive correlation between egg size and length of the prefeeding period and a subsequent decrease in the length of the feeding period to metamorphosis. Underwood (1974) used interspecific data to show that such assumptions are not valid. He was countered, in turn, by Steele (1977) who cited additional interspecific data to support Vance's original contention. However, Strathmann (1977) pointed out that interspecific variation in size at hatching and metamorphosis can confound interspecific relationships between egg size and developmental time so that correlations may appear is some taxa (including anuran amphibians) and not in others. Vance (1974) in fact stated that "crude interspecific comparisons are inadequate to reveal intraspecific evolutionary potential" and that such interspecific relationships "imply nothing about the egg-size - development-time relationship in any one species."

Any ecologically meaningful relationship between ovum size and development time must exist at the population level. Since the relative timing of developmental processes (the ratio of time between any two arbitrarily chosen stages) is the same within the tolerance range of a species (Bachman, 1969; Salthe and Mecham, 1974) any convenient stages can be used to compare rates between individuals in a population. In this study the time between gastrula (a convenient stage) and hatching (an ecologically important stage) is used. The data indicate that for all populations at all temperatures there is no relationship between ovum size and the time the embryo spends encapsulated. If the environment of a population gradually becomes colder, egg size might increase and developmental rate decrease but this latter effect cannot be ascribed to the change in egg size, and is most likely due to structural changes in enzyme catalysts involved in the developmental process (Sharpe and DeMichele, 1977) which are spuriously associated with egg size. Blaxter and Hempel (1963) in an interpopulational study with herring, showed that there was no relationship between time to hatching and egg size. Bell (1977), working with Triturus vulgaris, suggests that intrapopulationally the ova of larger females (presumably larger ova) hatch more rapidly. He was unable, however, to test his results statistically, and lumped the ova of different females together for the test.

The intrapopulational data presented here further contradict the claim that large ova have slower rates of development since, in fact, the offspring of females that produce larger eggs reach the feeding stage more rapidly (a consequence of increased hatchling size and respiratory rate; see below). Other interpopulational studies corroborate the intrapopulational data reported here. DuShane and Hutchinson (1944) reported that their eastern population of Ambystoma maculatum produced larger eggs than a midwestern population. The embryos of the eastern population developed more rapidly to the feeding stage, and the difference in rate (upon examination of their figure 1) becomes apparent only after the hatching stage is reached. In another interpopulational study, Pettus and Angleton (1967), working with the frog Pseudacris triseriata in northern Colorado, reported that the larger eggs produced by a high altitude population undergo more rapid development to matamorphosis.

The efficiency of development (temperature effects and egg size)

Materials stored in the yolk are rearranged into embryonic tissues. The efficiency of conversion is not perfect due to the loss of energy as heat from the system during the conversion process itself (growth metabolism). The conversion efficiency is further reduced as a result of maintenance requirements of the embryonic tissue (maintenance metabolism), and later in larval life as a result of the energy requirements of locomotion (active metabolism). As development proceeds increasing amounts of energy are lost as heat because of the increasing costs of maintenance and locomotion, and so efficiency is expected to drop. Nevertheless, by using well-defined starting and endpoints, the "gross efficiency" [=(calories contained in the embryo or larvae)÷(calories contained in the egg - calories not used)]; Blaxter, 1969 can provide important information about the implications of both egg size variability and temperature effects on embryonic development. Because the energy that remains stored in the yolk of amphibians at the hatching stage cannot be sorted out from the energy that is bound in embryonic tissue, the hatching stage is not suitable for measuring "gross efficiency." However, it is worth noting that in the data presented here, 1.45 (SD = 0.781) calories are used to reach the hatching stage. On the average this amounts to only 7% of the initial energy (figure 21). Fauré-Fremiet and Vivier du Streel (1921; as reported by Needham, 1931) found the value in the frog, Rana temporaria, to be 18%. Interspecific variability in this value is expected since amphibian embryos hatch at a variety of morphological stages. In addition, the variability is expected to be partly related to the reproductive mode of the species, as suggested by Crump and Kaplan (in prep.).

When the "gross efficiency" of development to the feeding stage is considered, the energy stored in the remaining yolk approaches zero, so that term drops out of the "gross efficiency" equation presented above. One is then essentially measuring the energy contained in a larva and dividing it by the energy initially contained in the egg. As has been shown, (see Results) there is a wide range of variability among the populations studied, with values ranging from 44.5 to 78.1%. [Fauré-Fremiet and Dragoiu (1923; as reported by Needham, 1931) found a value of 51.1% in the frog, Rana temporaria.] Intrapopulationally, the variability is positively correlated with variability in initial energy of the egg. More energy is not required to produce the larger larvae that result from larger eggs. Therefore the efficiency tends to increase with ovum size. Thus, an increase in ovum size (at least within the range of variability reported here) does not impose a measurable energetic drain on the developing embryos and therefore does not diminish the relative advantage of greater energy investments in individual offspring.

Licht (1971) realized that the adaptive significance of increased egg sizes in more northerly environments was not clear, and reasoned that "an investigation into the energy supply required for beginning life as a tadpole in deep cool water would help clarify the adaptive significance of large-yolked anuran eggs." Brown (1977), working with the largest yolked aquatic anuran egg (that of *Ascaphus trueii*), came to a similar conclusion. The variability in "gross efficiency" of development reported here is also positively correlated with temperature, which is made clear by an examination of figure 25. Therefore, an increase in egg size could compensate for the decrease in energetic efficiency of development in cold waters and account for the association of large eggs with cold environments at both the intra- and interspecific levels. Other data in the fish literature tend to support this view. Working with salmon embryos, Hayes and Pelluet (1945) also found efficiency increases from 42 to 59% when temperature changes from 0°to 16°C respectively. Wood (1932) found an increase from 54 to 63% between 3°C and warmer temperatures ranging between 7 and 12 C in salmon. Houde (1974) suggested that sea bream used yolk more efficiently at warmer temperatures. In an interpopulational study with herring, Blaxter and Hempel (1966) suggested that large eggs develop more efficiently at cold temperatures. Hamor and Garside (1977) found that in salmon efficiency increases at warmer temperatures after hatching.

As temperature increases beyond a critical point one expects efficiency to drop off as enzyme systems collapse. Ryland and Nichols (1967) used as a measure of efficiency the rate of increase in length divided by the rate of disappearance of yolk between the hatching and feeding stages. They found an optimum value at an intermediate temperature in plaice prolarvae. Jones (1972) and Marr (1966), in *Scophthalmus* and salmon respectively, also found optimal temperatures. When the

Ryland and Nichols efficiency measure is applied to the data presented here, an increase in efficiency between 10° and 20°C is found in 3 out of 4 populations. In order to show whether there is an optimum value, more intermediate temperatures would have to be studied. Nevertheless, the increase at 20°C shows that if an optimum does exist (which seems possible since *A. tigrinum*, the most cold-adapted species, showed no increase in efficiency at 20°C), it would still be at a temperature warmer than 10°C.

The effects of fluctuating temperatures

It could be argued that the effects of warm or cold temperature regimes on developmental characteristics do not obtain in nature since most organisms do not live under constant temperature conditions. As has been shown in studies on insects (see Howe, 1967, and Sweeney and Schnack, 1977, for review) the effects of fluctuating temperature can be profound. For example, if the mean temperature in a fluctuating temperature environment is below the temperature at which no development takes place, development can still occur due to the effects of the warmer temperature (if it is above the zero development temperature). While Howe (1967) felt that available evidence was too inconclusive to prove that alternating temperatures either stimulate or retard development of insect eggs, Hagstrum and Hagstrum (1970) felt that studies generally indicate that insects develop more rapidly in an alternating environment when the maximum and minimum temperatures are within the optimal range of development for the organism. This would lead to profound ecological effects as has been shown by Siddiqui and Barlow (1972) in *Drosophila melanogaster*. They found that as a result of more rapid development and faster attainment of sexual maturity in a fluctuating temperature environment, r_{max} was significantly increased.

The alternating temperature environment was used in this study as a possible control for this problem, and also to allow any unusual effects to appear. For example, metabolic compensation in fluctuating temperature environments may in itself entail extra energy expenditure and thus lower the overall efficiency of the process. The data indicate, however, that size, energy content, time to hatching and feeding stages, and the efficiency of the developmental process are not affected in any unusual way in any of the four populations.

Herreid and Kinney (1967) found developmental rates in *Rana sylvatica* to be the same as the rate predicted for the mean of an environment that fluctuated between 10°and 16.1°C on a 12 h. cycle. The data presented here (where temperature fluctuations were greater) are in accordance with their findings. The non-intermediate values for developmental time in alternating environments (shown in figure 20) are expected if the rate temperature function is assumed linear (Bachman, 1969). The interpolated value for developmental time at the mean temperature of 15°C lies on a hyperbolic curve. Buchanan (1940; criticized by Ryan, 1941) claimed to have found an acceleration when environments alternated between 5°and 21°C on either a 12 h. or 24 h. cycle but not on a 6 h. cycle. Such extreme fluctuations in temperature would be very unusual occurrences at least in the populations studied. The generally intermediate values for parameters measured here are taken to bolster the view that the effects of warm and cold constant temperatures on development in these cold adapted *Ambystomas* are meaningful even though under natural conditions temperatures fluctuate.

Early larval growth rates and egg size

Growth rates of *Ambystoma* larvae have been shown to depend on temperature, food quality and the density of individuals in artificial environments (Hutchinson and Hewitt, 1935; Stewart, 1956; Wilbur 1971 and 1972; Wilbur and Collins, 1973). None of these studies, or any others in amphibians, considers the effects of differences between offspring of specific females. The data on variability in hatchling and feedling sizes and subsequent growth during the first three weeks following the onset of feeding, establish the potential importance of inter-individual egg size variability. In their application of the Gompertz function ($Wt = Wo \exp \left[(A/a)(1-\mathrm{expat}) \right]$; where Wt = size of larvae after time t, Wo = size of larvae at start of experiment, A = size specific growth rate, a = damping effects on growth) Wilbur and Collins (1973) stated that "the variation in size at hatching is too small to account for the great variation later in the larval period." This is equivalent to saying that if *A* and *a* are constants, the intrinsic variation in *Wt* will not be greater than that in *Wo*. As indicated by the slopes being greater than 1.0 in figure 29, *A* tends to be positively related to *Wo*. This would tend to increase the variability in *Wt* and result in the presumed advantages of larger size being projected into and exaggerated at later stages. Lawlor (1976) found a similar relationship in the isopod *Armadillidium vulgare*. The skew in larval size variability, that Wilbur and Collins (1973) found to increase with increasing density in their experimental pens would, however, be caused by still other factors. Nevertheless, one would predict from this work that the larvae that comprise the tail of their positively skewed distributions would almost always be derived from the clutches of females that produced the largest eggs. Wilbur and Collins also maintained that the variability in hatching times within their experiments is not critical but would be important in nature. Variability in time of oviposition (and subsequent hatching times) will tend to confound any general predictive model of amphibian larval growth and metamorphosis. If one assumes, however, that over the course of several breeding seasons timing of oviposition tends to normalize, then from an evolutionary point of view hatchling size (egg size) determines the rate of differentiation to the feeding stage and the subsequent onset of post-feeding growth (which is the first opportunity for the larvae to increase in mass). If <u>t</u> is considered as the time since the onset of feeding, then at any given sampling of a population of larvae that were all deposited at the same time, <u>t</u> is also positively correlated with <u>Wo</u>. Thus the "small" variation in hatchling sizes is amplified through time by both variation in size-specific growth rates and time of actual growth. Since in natural populations the variation in <u>Wo</u> would have a large female-specific component selection can act quite strongly. Clutch size would be determined not only by body size constraints but by developmental modal constraints.

If competition is important in nature, then opportunities for food capture and growth will tend to favor larger larvae, resulting in the observed skewed body size distributions (Wilbur and Collins, 1973). If predation is an important factor, larger larvae will more likely avoid capture (although the opposite is possible in some unlikely circumstances). If the ephemeral nature of the pond is important, larger larvae that are growing faster may be able to metamorphose before the pond dries. Although if ponds are long-lasting in some years, then slower-growing larvae may metamorphose at an absolutely larger size, being perhaps better able to survive terrestrial life to first reproduction than their early metamorphosed sibs (Shoop, 1974).

A large gap exists in our knowledge concerning factors that influence larval population sizes in nature. It is probable that while large body size is most likely advantageous to a larva, under certain circumstances it may be disadvantageous. From the point of view of the female parent, if mortality is unrelated to sizes of larvae, then the production of many small eggs would be most likely to result in her leaving successful offspring. The trade-off between egg size and egg number which has been demonstrated within some of these animals, and the large amount of variability that exists in the size of eggs and offspring that are produced, tend to indicate that successful reproduction can be accomplished in a variety of ways within the same population. Some females succeed by producing fewer large eggs, others by producing more but smaller eggs.

FOR FUTURE STUDY

Two major questions are raised by the present study. How many environmental factors need to be considered in order to explain the types of variability found. Multifactor analyses of environments must be undertaken. Factors that are both larval-size independent and dependent must be considered simultaneously, because no doubt both are important. Second, the fidelity of a female to a particular strategy needs to be determined. Does egg size vary from year to year in a given female as much as it varies between females within a year? Shrode and Gerking (1977) have recently shown, for example, that egg size is inversely related to temperature within single females in the desert pupfish. Kerfoot (1974), on the other hand, explained seasonal (temperature associated) egg size changes in a cladoceran as being an adaptation against predation, implying a strong genetic component. In other words, is a single female a-jack-of-all-environments by having a variable strategy or are strategies fixed with yearly environmental variability and extended life resulting in successful lifetime reproduction?

SUMMARY AND CONCLUSIONS

1.) Within all five Ambystoma populations studied, different females produce ova of significantly different sizes. The differences can be so large that one female in a population can be producing ova that have twice the volume as those of another in the same population at the same time.

2.) A large amount of interspecific data on salamander body volume, clutch size, ovum size and clutch volume is summarized. These data are used to construct allometric null hypotheses which allow for the subtraction of body size effects in an analysis of body size constraints on clutch parameters. The negatively allometric relationship that has been discovered between female body cavity volume and clutch volume indicates that interspecifically, all things being equal, the females of a large-bodied species are expected to have a smaller proportion of their body volumes occupied by a clutch mass than those of a smaller-bodied species.

3.) The usefulness and necessity of first determining how a particular reproductive trait is expected to change with body size is made clear by the following example: females of two populations studied, A. tigrinum and A. opacum-N.J., have an average of 13.6% of their body volumes occupied by ova. The females of the A. opacum-N.J. population do not deviate from what is expected for salamanders of their particular size. Female A. tigrinum, however, are producing 1.9 times as large a clutch mass as predicted for salamanders of their respective size. The females in this latter population are shown to be trading off ovum size for ovum number.

An ecological interpretation of these phenomena is presented.

4.) The offspring of some females in a single population can begin life at the same time as those of other females with half the energy and material. There are significant differences both within and between populations with reference to dry weight-specific energy content of ova, meaning that a constant value cannot be assumed to apply to all individuals.

5.) Developmental rate to the hatching stage is not affected by ovum size intrapopulationally, but larger ova develop into feedlings in a shorter time period.

6.) The absolute difference in energy content of ova that exists among the females within a population is generally maintained by their larvae at both the hatching and feeding stages. A relationship between respiratory rate and larval size can therefore be used to explain ovum size related differences in developmental rate.

7.) An alternating temperature environment is used to control for fluctuating temperatures in nature. The data generally show that development at constant temperatures is not qualitatively different from that in an alternating temperature using a variety of criteria.

8.) The different effects of warm and cold temperatures on development are discussed and among other things the efficiency of development is shown to increase at warmer temperatures. Efficiency is also shown to increase with increasing ovum size.

9.) Variation in developmental times and initial size are related through a Gompertz function which shows that potential advantages of large ovum size to an offspring increase after feeding commences, besides being maintained throughout the pre-feeding period.

10.) A continuum of strategies is shown to exist even within one population. A female that produces larger eggs may have to produce fewer if body size is constraining as in _A. tigrinum_. These larger eggs would be superior to smaller ones in a competitive and predator-rich environment. Smaller eggs, however, in allowing more to be produced, would enable a female to be equally successful if environmental conditions are primarily density-independent relative to the embryos and larvae. It is suggested that a continuum of strategies may be maintained by a complex environment where density dependent and independent factors work either simultaneously, randomly on a seasonal basis, or both.

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