THE ROLE OF TISSUE DENSITY IN THE TERRESTRIALISM OF AMBYSTOMA OPACUM

BY

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THE ROLE OF TISSUE DENSITY IN THE TERRESTRIALISM OF *AMBYSTOMA OPACUM*

A Research Paper

Presented to

the Graduate Council of

Austin Peay State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Philip Ross Chadwick

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To the Graduate Council:

I am submitting herewith a research paper written by Philip Ross Chadwick entitled "The Role of Tissue Density in the Terrestrialism of Ambystoma opacum." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

David H. Snyder
Major Professor

Accepted for the Council:

Dean of the Graduate School

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I. INTRODUCTION

In the transition from the aquatic to the terrestrial mode of life by amphibians, the problem of the conservation of body moisture presented itself. To an aquatic species constantly bathed in water, this offers no difficulty. But a terrestrial species is obviously exposed to the effects of evaporation for long periods of time and, therefore, must replace the surface moisture lost through evaporation from its body reserves. Thorson and Svihla (1943) recognized that in order to endure a comparatively dry habitat and to cope with the inevitable loss of body water a terrestrial amphibian must possess some adaptations not present in aquatic forms. The assumed existence of such an adaptation served as the basis for this study.

Review of the Literature

Thorson (1955) stated that despite the apparent lack of a highly effective water-conserving mechanism in amphibians as a group, marked differences exist between species in their degree of dependence on water. Some species are restricted to water throughout life, a few have been completely emancipated from open water, and the remaining species are distributed between those two extremes.

The relatively greater tolerance to dehydration shown by terrestrial amphibians has led to speculation as to possible physiological and behavioral adjustments which may aid those forms in survival. Moore (1964) reported there has been considerable controversy as to whether the skins of terrestrial amphibians are or are not less permeable to water than those of aquatic species. More recently, Prosser (1973) stated that the permeability of amphibian skin to water is less in terrestrial than in aquatic species.

Spight (1967), Thorson (1955), Thorson and Svihla (1943), Ray (1958), Littleford, et al. (1947) and Jørgensen (1950) have all conducted studies of the relationship of the amphibian to its environment with regard to water requirements. Most experiments concerned with water relations in amphibians have been done on the aspect of dehydration in plethodontid salamanders.

Littleford, et al. (1947) conducted histological studies of amphibian skin. They concluded, on the basis of the absence of any major differences between the skin of terrestrial and aquatic species and in light of previous research, that the skin is not the adaptation permitting survival of certain terrestrial salamanders. Thorson and Svihla (1943) found that terrestrial species of anurans tolerated desiccation better than did species from aquatic habitats.

Ray (1958) determined that the greatest water loss is generally tolerated by those species of salamanders that live in the driest habitat. He also stated that this correlation is evident at the family level, less evident at the generic level and only occasionally present at the species level.

Bently (1966) reported that adaptations of amphibians to arid environments was accomplished through several means. Desert amphibians may show physiological acclimatization with respect to their ability to tolerate high temperature. Their ability to absorb water through the skin may be an advantage, as well as might be the ability of different amphibians to withstand desiccation. Amphibians may utilize a tolerance for urea to allow nitrogen storage in a relatively nontoxic form, to be excreted at some time when more water is available.

<u>Statement of the Problem</u>

Briggs (1939) observed in <u>Rana pipiens</u> that from fertilization to the beginning of neural fold development the density of the embryo decreased from 1.1065 to 1.050. During the period of closure of the neural folds and elongation of the embryo, the density increased suddenly to 1.085. Then during subsequent development to hatching, it decreased steadily to 1.048. During neurulation the marked increase in the density is accompanied by rapid changes in structure; the embryo elongates, the perivitelline space enlarges, and the archenteron decreases in volume.

Brown (1942), considering a series of determinations of densities of embryonic tissues by Brown, Hamburger, and Schmitt (1941), reported that the prospective neural tissue of <u>Ambystoma opacum</u> had a markedly lower density than the corresponding tissue of <u>A. maculatum</u>, <u>A. t. tigrinum</u> or <u>Rana palustris</u>. This lower density, he speculated, may indicate a greater water holding capacity of neural tissue of <u>A. opacum</u> than is true for the same tissue of species which lay their eggs in water, therefore constituting an adaptation to terrestrialism.

Rappaport (1954) reported that during development, differentiation is often accompanied by regional differences in rate of growth. The fact that one embryonic region may grow more rapidly than another may be attributed to either cellular movement into the rapidly growing region or to local increase in tissue volume or to a combination of the factors. In the absence of cellular contributions from other regions, local increase in tissue volume must be considered as accomplished without significant gain of solid materials in the precirculatory stages. In this case, the tissue volume gain, whether or not it is accompanied by an accelerated rate of cell division, must be made up by an increase in water content. That increased water content lowers the tissue density is substantiated by Tsai and Lin (1939) when they observed that the electric stimulation of frog gastronemius increased the water content of the tissue thereby resulting in a lower density of the solid tissue.

There are some possible species relationships which perhaps should be considered. Noble and Brady (1933) and Anderson (1961) found the courtship behavior of A. opacum and A. maculatum to be essentially alike, whereas Salthe (1967) described A. talpoideum and A. opacum as having the same courtship. According to Tihen (1958) A. opacum and A. talpoideum probably arose together from an A. maculatum-like stock in the southeastern United States. Thus evidence seems to favor a close phylogentic relationship between A. opacum and A. maculatum.

Salthe (1963) found A. opacum eggs, although they are laid on land, to be essentially unchanged from the aquatic type. The pattern of infrequent breeding seemed typical of both A. opacum and A. maculatum. He suggested that this leaves only the timing of the breeding migration as a significant departure from other species of the family.

That writers mentioned above have placed emphasis upon drawing comparisons between A. opacum and other family members, indicates that there must be some importance to an adaptation to permit its terrestrial mode of reproduction.

Purpose of the Study

The development of terrestrial amphibians necessitated some adaptation to enable them to survive considerable exposure to evaporation. If the neural tissue of the embryos of A. opacum has a lower density, then the question arises as to whether this lower density exists in the adults. If it does, then this physical adaptation may aid in the ability of the adult female to brood her eggs on land.

The Ambystomatidae were chosen for study because of the departure of A. opacum from the normal aquatic mode of reproduction in this family. Unlike the other family members which lay their eggs in water in the late winter or early spring, A. opacum lays its eggs in the fall, on land. After egg deposition, the female remains with the eggs until the nesting site is flooded by fall or winter rains, which may not occur for several weeks.

Previous work concerning desiccation studies has not included members from the Ambystomatidae. Data corrobarating the contention that A. opacum, as an adult, is more terrestrial than other family members is not available in the literature, excepting a statement by Bishop (1941) that most writers agree that it is found in drier situations than most other species of the genus.

Other workers such as Spight (1967), Thorson (1955), Thorson and Svihla (1943), Ray (1958), Littleford, et al. (1947) and Jørgensen (1950) have attempted to explain the ability of certain amphibians to exist in arid environments on the basis of reduced permeability of the skin to water, or on their ability to concentrate urine. The purpose of this study was to investigate differing body densities among certain species of amphibians, and to interpret the observed pattern of variation in terms of possible significance as an adaptation to terrestrialism and the abilities to resist desiccation.

II. METHODS AND MATERIALS

The study utilized the following members of Ambystomatidae: *Ambystoma t. tigrinum*, *A. texanum*, *A. talpoideum*, *A. jeffersonianum*, *A. maculatum* and *A. opacum*. The specimens used were obtained commercially. Ten individuals of each species, except for *A. opacum* in which case eight were used, were utilized in the study.

Tissue density determinations were made by a floatation procedure in solutions of gum arabic. The procedure of the study necessitated the use of a series of known density solutions in which to immerse the tissues under study to determine the density of each tissue. The solute had to be a complex molecule so that osmosis did not occur between the solution and the tissue, and for this reason gum arabic with a molecular weight of about 240,000 was used.

Stock gum arabic solution was prepared by dissolving the powder in distilled water. A few crystals of thymol were added to prevent the growth of molds and bacteria. The stock solution was filtered through sterile cotton, with the aid of a vacuum pump, to remove solid particles. Working solutions were then prepared in a range of densities from 1.010 to 1.110, in 0.004 increments, and stored in stoppered glass jars.

Density determinations were made in accordance with a procedure suggested by Briggs (1939). To verify the repeatability of the determinations a neural tissue sample was re-checked.

The specimens were maintained in plastic boxes with water-saturated peat moss for eight days to assure equally hydrated states before death. The specimens were inadvertently killed by freezing, as a result of a malfunction of the environmental chamber in which they were housed. All specimens were then placed in containers of distilled water and frozen within twenty-four hours of their death. Fifteen days lapsed between the death and final density determinations.

Density determinations were made for the following; the whole specimen, specimen less its viscera, neural tissue, muscle tissue and liver tissue. The whole specimen was prepared by thoroughly rinsing it in distilled water. It was then immersed into the gum arabic solutions. With each solution change, the specimen and the glassware involved were rinsed with distilled water. A median ventral incision was then made through the body wall from the cloaca to the lower jaw bone, and all parts of the viscera removed. The specimen without its viscera was then kept in an iced distilled water bath until used. The liver was removed from the extracted viscera and maintained in the same manner. For neural tissue the sciatic nerve was removed after all determinations were made on the specimen less its viscera. The tail was incised behind the cloaca, and its skin removed to reveal the caudal muscles from which a sample was taken. All denstiy determinations were made of all the appropriate tissues of a particular individual before proceeding to the next individual.

Since the specimens of the study were frozen by accident, the density determination procedure was made on a sample of Desmognathus fuscus. Half of the sample was frozen and was compared to the remaining individuals to see if there was a difference between fresh and frozen tissue samples. D. fuscus was used because it was easy to acquire. Density determinations were made for whole specimen, specimen less its viscera and neural tissue. No significant difference occured between fresh and frozen samples of this species.

The data collected were statistically analyzed. Means and standard deviations were calculated for each tissue of each taxon, and the Student's t-test was used in assessing the significance of the observed differences in particular tissue densities among the species. The statistical findings then served as the basis for conclusions concerning the questions which the study was designed to answer.

III. RESULTS

Table I presents the mean density values and the standard deviations of the tissues studied. Tables II through VI present the Student's t-test results and their corresponding alpha values for the various species-tissue comparisons made during the study.

The Student's t-test was used in assessing the significance of the observed differences among the mean densities of the tissues studied. When Ambystoma opacum was compared to each of the other five species in the study, for all twenty-five possible tissue-species test combinations, all but six of the comparisons yielded significantly different values at the 99.9% level of confidence (Tables II thru VI). Four of the five comparisons of neural tissue of A. opacum with the same tissue of other species yielded insignificant results. Only A. talpoideum had neural tissue differing significantly in density from that of A. opacum. The remaining two comparisons of A. opacum tissue density not differing significantly from the densities of the same tissues of other species were with the muscle tissue of A. jeffersonianum and the liver tissue of A. maculatum.

An examination of Table II shows that all but three comparisons of the comparisons of densities of whole specimens were significantly different at the 95% level of confidence. Those insignificant comparisons were of A. t. tigrinum with A. maculatum, A. t. tigrinum with A. texanum, and A. maculatum with A. texanum.

Table III presents the results of comparisons of the tissue densities of the specimens less their viscera. All but three of those

TABLE I

MEANS AND STANDARD DEVIATIONS OF THE DENSITIES OF FIVE TISSUES OF SIX SPECIES OF AMBYSTOMATID SALAMANDERS

Name of Specimen	Number of Specimen	Whole Specimen	Less Viscera	Neural Tissue	Muscle Tissue	Liver Tissue
Ambystoma jeffersonianum	10	1.0536 ±0.0021	1.0568 ±0.0018	1.0341 ±0.0018	1.0432 ±0.0017	1.0427 ±0.0017
Ambystoma t. tigrinum	10	1.0348 ±0.0017	1.0413 ±0.0015	1.0336 ±0.0010	1.0424 ±0.0019	1.0252 ±0.0014
Ambystoma talpoideum	10	1.0429 ±0.0013	1.0453 ±0.0016	1.0550 ±0.0015	1.0373 ±0.0017	1.0262 ±0.0018
Ambystoma maculatum	10	1.0378 ±0.0018	1.0404 ±0.0015	1.0353 ±0.0017	1.0402 ±0.0019	1.0348 ±0.0018
Ambystoma texanum	10	1.0394 ±0.0023	1.0418 ±0.0021	1.0349 ±0.0014	1.0420 ±0.0021	1.0263 ±0.0021
Ambystoma opacum	8	1.0303 ±0.0021	1.0325 ±0.0021	1.0431 ±0.0015	1.0460 ±0.0017	1.0344 ±0.0019

TABLE II

STUDENT'S T-TEST VALUES AND ALPHA VALUES OF SIGNIFICANCE LEVELS FOR TISSUE DENSITY IN THE WHOLE SPECIMEN OF SIX AMBYSTOMATID SALAMANDERS

	Ambystoma t. tigrinum	*Ambystoma talpoideum*	*Ambystoma maculatum*	*Ambystoma texanum*	*Ambystoma opacum*
Ambystoma jeffersonianum	$\alpha < 0.001$ 14.86	$\alpha < 0.001$ 13.55	$\alpha < 0.001$ 17.90	$\alpha < 0.001$ 14.13	$\alpha < 0.001$ 23.47
Ambystoma t. tigrinum		$\alpha < 0.001$ 6.55	$0.5 > \alpha > 0.4$ 0.84	$0.4 > \alpha > 0.2$ 1.10	$\alpha < 0.001$ 9.23
Ambystoma talpoideum			$\alpha < 0.001$ 7.17	$\alpha < 0.001$ 4.03	$\alpha < 0.001$ 15.79
Ambystoma maculatum				$0.1 > \alpha > 0.05$ 1.77	$\alpha < 0.001$ 8.20
Ambystoma opacum					$\alpha < 0.001$ 8.66

TABLE III

STUDENT'S T-TEST VALUES AND ALPHA VALUES OF SIGNIFICANCE LEVELS FOR TISSUE DENSITY IN THE SPECIMEN LESS ITS VISCERA OF SIX AMBYSTOMATID SALAMANDERS

	Ambystoma t. tigrinum	*Ambystoma talpoideum*	*Ambystoma maculatum*	*Ambystoma texanum*	*Ambystoma opacum*
Ambystoma jeffersonianum	$\alpha < 0.001$ 20.38	$\alpha < 0.001$ 14.83	$\alpha < 0.001$ 21.48	< 0.001 17.00	$\alpha < 0.001$ 26.24
Ambystoma t. tigrinum		$\alpha < 0.001$ 5.65	$0.2 > \alpha > 0.1$ 1.34	$0.5 > \alpha > 0.4$ 0.69	$\alpha < 0.001$ 10.19
Ambystoma talpoideum			$\alpha < 0.001$ 7.04	$\alpha < 0.001$ 4.13	$\alpha < 0.001$ 14.62
Ambystoma maculatum				$0.1 > \alpha > 0.05$ 1.83	$\alpha < 0.001$ 9.23
Ambystoma opacum					$\alpha < 0.001$ 9.41

TABLE IV

STUDENT'T T-TEST VALUES AND ALPHA VALUES OF SIGNIFICANCE LEVELS FOR TISSUE DENSITY IN THE NEURAL TISSUE OF SIX AMBYSTOMATID SALAMANDERS

	Ambystoma t. tigrinum	*Ambystoma talpoideum*	*Ambystoma maculatum*	*Ambystoma texanum*	*Ambystoma opacum*
Ambystoma jeffersonianum	$0.5>\alpha>0.4$ 0.86	$\alpha<0.001$ 32.62	$0.1>\alpha>0.05$ 1.88	$0.2>\alpha>0.1$ 1.42	$0.9<\alpha$ 0.11
Ambystoma t. tigrinum		$\alpha<0.001$ 36.79	$0.02>\alpha>0.01$ 2.79	$0.05>\alpha>0.02$ 2.40	$0.4>\alpha>0.2$ 0.88
Ambystoma talpoideum			$\alpha<0.001$ 27.64	$0.01>\alpha>0.001$ 3.02	$\alpha<0.001$ 28.85
Ambystoma maculatum				$0.9>\alpha>0.5$ 0.56	$0.2>\alpha>0.1$ 1.57
Ambystoma opacum					$0.4>\alpha>0.2$ 1.15

TABLE V

STUDENT'T T-TEST VALUES AND ALPHA VALUES OF SIGNIFICANCE LEVELS FOR TISSUE DENSITY IN THE MUSCLE TISSUE OF SIX AMBYSTOMATID SALAMANDERS

	Ambystoma t. tigrinum	*Ambystoma talpoideum*	*Ambystoma maculatum*	*Ambystoma texanum*	*Ambystoma opacum*
Ambystoma jeffersonianum	$0.4>\alpha>0.2$ 0.97	$\alpha<0.001$ 7.74	$0.01>\alpha>0.001$ 3.67	$0.2>\alpha>0.1$ 1.35	$0.01>\alpha>0.001$ 3.49
Ambystoma t. tigrinum		$\alpha<0.001$ 6.31	$0.05>\alpha>0.02$ 2.54	$0.9>\alpha>0.5$ 0.42	$\alpha<0.001$ 4.17
Ambystoma talpoideum			$0.01>\alpha>0.001$ 3.71	$\alpha<0.001$ 5.50	$\alpha<0.001$ 10.76
Ambystoma maculatum				$0.01>\alpha>0.001$ 2.98	$\alpha<0.001$ 6.15
Ambystoma texanum					$\alpha<0.001$ 4.33

TABLE VI

STUDENT'S T-TEST VALUES AND ALPHA VALUES OF SIGNIFICANCE LEVELS FOR TISSUE DENSITY IN THE LIVER TISSUE OF SIX AMBYSTOMATID SALAMANDERS

	Ambystoma t. tigrinum	Ambystoma talpoideum	Ambystoma maculatum	Ambystoma texanum	Ambystoma opacum
Ambystoma jeffersonianum	$\alpha < 0.001$ 27.74	$\alpha < 0.001$ 12.83	$\alpha < 0.001$ 10.56	$\alpha < 0.001$ 20.48	$\alpha < 0.001$ 12.00
Ambystoma t. tigrinum		$0.2 > \alpha > 0.1$ 1.38	$\alpha < 0.001$ 13.34	$0.2 > \alpha > 0.1$ 1.40	$\alpha < 0.001$ 10.23
Ambystoma talpoideum			$\alpha < 0.001$ 10.53	$0.9 < \alpha$ 0.11	$\alpha < 0.001$ 7.76
Ambystoma maculatum				$\alpha < 0.001$ 9.76	$0.9 > \alpha > 0.5$ 0.64
Ambystoma texanum					$\alpha < 0.001$ 7.14

comparisons were significantly different at the 95% level of confidence. Those not significant were of A. t. tigrinum with A. maculatum, A. t. tigrinum with A. texanum, and A. maculatum with A. texanum.

Table IV presents the results of comparisons of neural tissue densities. An examination of those results reveals that of twenty-five comparisons, eight were not significantly different at the 95% confidence level. Those eight were comparisons of A. jeffersonianum with A. t. tigrinum, A. jeffersonianum with A. maculatum, A. jeffersonianum with A. texanum, A. jeffersonianum with A. opacum, A. t. tigrinum with A. opacum, A. maculatum with A. texanum, A. maculatum with A. opacum, and A. texanum with A. opacum.

Table V presents the comparisons of muscle tissues. Only three comparisons observed were not above the 95% level. These were A. jeffersonianum with A. t. tigrinum, A. jeffersonianum with A. texanum, and A. t. tigrinum with A. texanum.

Table VI presents the comparisons for liver tissue from the five species studied. The four comparisons not significant above the 95% level were: A. t. tigrinum with A. talpoideum, A. t. tigrinum with A. texanum, A. talpoideum with A. texanum, and A. maculatum with A. opacum.

IV. DISCUSSION AND CONCLUSIONS

In ranking the mean density values, the order from lowest density to highest density for whole specimen was as follows; Ambystoma opacum, A. maculatum, A. t. tigrinum, A. texanum, A. talpoideum and A. jeffersonianum. When the specimen less its viscera was ranked, the same order was observed. When the remaining mean tissue densities were ranked, much shifting of positions occurred among the species. The neural densities found A. t. tigrinum with the least density followed by A. jeffersonianum and A. opacum (tied for second place), A. texanum, A. talpoideum and A. maculatum. The muscle tissue densities from least to greatest were as follows; A. talpoideum, A. maculatum, A. texanum, A. t. tigrinum, A. jeffersonianum and A. opacum. Liver tissue rankings began with A. t. tigrinum, followed by A. talpoideum, A. texanum, A. opacum, A. maculatum and A. jeffersonianum.

An examination of the Student's t-test values revealed that twenty of the twenty-five comparisons between A. opacum and the other species studied differed at or above the 95% significance level. The least significance of difference between A. opacum and the other species occurred in the case of neural tissue. The only other comparison not significantly different at the 95% level of confidence was with A. maculatum for liver tissue.

A factor which may or may not have had a result in the outcome of the study was that of the ecological variation of the specimens. Since the specimens were obtained commercially, it was not known if all of the individuals came from the same site or even from the same general ecological situation.

The idea that perhaps the embryo is the stage in the life cycle subjected to more desiccation stress than the adult should not be overlooked. Whereas the adult can change its ecological situation and seek more moist environments, the embryo is enclosed within the capsules of the egg until hatching. It is reasonable to assume that if hatching ocurred, then the breeding site must contain adequate water to fill the breeding depression. Whether or not an individual nest is flooded depends upon the effectiveness of the breeding strategy of the female in placing the nest within the boundary of a subsequently flooded site. Also, I have observed that during the drier portions of the nesting season, the egg may be greatly reduced in size because of lower water content. The loss of water obtained from the leaf litter of the nest not only reduces the egg fluid content, but it raises the concentration levels of the salts and other materials in which the embryo is contained.

As Ray (1958) observed in his study, the correlation between water economy and the habitat is only occasionally present at the species level. The six members of this study reveal density values of little variation between the specimen and species. The difference between species is not great enough to conclude that density of tissues plays a role in the terrestrial habits of certain salamanders.

V. SUMMARY

The following members of Ambystomatidae were studied to determine if a correlation existed between tissue density and the terrestrial habits of the salamanders: *Ambystoma jeffersonianum*, *A. t. tigrinum*, *A. talpoideum*, *A. texanum*, *A. maculatum* and *A. opacum*. *A. opacum* differed from the other species in its pattern of reproduction, using a terrestrial method rather than the usual aquatic family method. The purpose of this study was to investigate the role of tissue density in this life style.

Density determinations were made by a floatation procedure in solutions of gum arabic. The tissues studied were the whole specimen, the specimen less its viscera, neural tissue, muscle tissue and liver tissue. Density values were obtained and a statistical analysis was conducted on the data.

Some of the tissues for *A. opacum* were of lower density than the same tissues from the other species. However the fact that nearly everything differed from nearly everything else made it impossible to detect any logical pattern of significanct differences between *A. opacum* and other members of this genus. I conclude that there is no evidence to indicate that *A. opacum* has a more terrestrial life style because of lower tissue density.

LITERATURE CITED

Anderson, J. D. 1961. The courtship behavior of Ambystoma macrodactylum croceum. Copeia 1961: 132-139.

Bently, P. J. 1966. Adaptations of Amphibia to arid environments. Science 152: 619-623.

Bishop, S. C. 1941. The salamanders of New York. New York State Mus. Bull. 324: 1-365.

Briggs, R. W. 1939. Changes in the density of the frog embryo (Rana pipiens) during development. J. Cell. Comp. Physiol. 13: 77-89.

Brown, M. G. 1942. An adaptation in Ambystoma opacum embryos to develment on land. Am. Nat. 76: 222-223.

Brown, M. G., Vitkor Hamburger, and F. O. Schmitt. 1941. Density studies on amphibian embryos with special reference to the mechanism of organizer action. J. Exp. Zool. 88: 353-372.

Jørgensen, C. B. 1950. The amphibian water economy, with special regard to the effect of neurohyposeal extracts. Acta Physiol. Scand. 22; Supplementum 78. 79p.

Littleford, R. A., W. F. Keller, and N. E. Phillips. 1947. Studies on the vital limits of water loss in the plethodont salamanders. Ecology 28: 440-447.

Moore, J. A. 1964. Physiology of the amphibia. Academic Press, New York. 654p.

Noble, G. K. and M. K. Brady. 1933. Observations on the life history of the marbled salamander, Ambystoma opacum Gravenhorst. Zoologica 11: 89-132.

Prosser, C. L. 1973. Comparative animal physiology, Third edition. W. B. Saunders Co., Philadelphia. 1024p.

Rappaport, Raymond, Jr. 1954. The uptake of water during development of amphibian tissues. J. Exptl. Zool. 127: 27-53.

Ray, Carleton. 1958. Vital limits and rates of desiccation in salamanders. Ecology 39: 75 83.

Salthe, S. N. 1963. The egg capsule in the amphibia. J. Morph. 11: 161-171.

Salthe, S. N. 1967. Courtship patterns and the phylogeny of the urodeles. Copeia 1967: 100-117.

Spight, T. M. 1967. The water economy of salamanders: water up-take after dehydration. Comp Biochem. Physiol. 20: 767-771.

Thorson, T. B. 1955. The relationship of water economy to terrestrialism in amphibians. Ecology 36: 100-116.

Thorson, T. B. and Arthur Svhila. 1943. Correlation of the habits of amphibians with their ability to survive the loss of body water. Ecology 24: 374-381.

Tihen, J. A. 1958. Comments on the osteology and phylogeny of ambystomatid salamanders. Bull. Fla. State Mus. 3: 1-50.

Tsai, Chaia and Chun-Yu Lin. 1939. The density of animal tissue. Chinese J. Physiol. 14: 39-50.