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Early Development of the Gulf Coast Toad, *Bufo valliceps* Wiegmann

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Most taxonomic investigations on anuran larvae have lacked the precision and clarity required for critical, comparative studies of tadpoles of different species. Published descriptions of anuran tadpoles have been based principally on samples collected in the field. Larvae rarely have been reared through metamorphosis or obtained experimentally from known parents to insure positive identification. Verification of descriptions of tadpoles of uncertain parentage has long been needed.

Some investigators have actually admitted describing only those tadpoles that are "mature," the implication being that "immature" tadpoles are of no or limited taxonomic importance. "Half-grown larvae," as Wright (1929) stated, ". . . are often quite abnormal in the usual characters used in larval descriptions." It thus follows that an investigator will be unable to identify the tadpoles if he happens to obtain a series of "immature" individuals. This failure to consider ontogenetic changes of "diagnostic characters" of a species has resulted in considerable taxonomic confusion.

Moreover, the significance of such terms as "mature," "immature," "half-grown," and "fully developed" as applied to tadpoles is highly

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questionable. The various stages of early development[1] must be precisely and unequivocally defined. As Orton (1953, 1955a, 1955b) stressed, comparative studies of embryos and tadpoles of species must be based on unambiguously described, equivalent growth stages.

Embryologists have been prolific in determining and recording the early developmental stages in several anuran species. These descriptive series aid the embryologist in "speaking a common language" in research. Pollister and Moore (1937) defined and illustrated 23 embryonic stages of *Rana sylvatica;* Weisz (1945) presented a series of 23 identifiable periods in the early development of *Xenopus laevis;* Eakin (1947) illustrated the development of *Hyla regilla* from the neural fold stage to the first appearance of the operculum; and Conte and Sirlin (1952) provided a photographic reference series of 25 embryonic stages of *Bufo arenarum.* The early development of *Rana pipiens* has been described in two works. Shumway (1940) described and illustrated 25 perfeeding stages, and Taylor and Kollros (1946) completed the series with a description of the larval changes from the first feeding stage through metamorphosis.

These developmental series, among others, were prepared especially for embryological research, but can be utilized as a framework upon which to erect a "taxonomic" developmental series. The descriptions by Taylor and Kollros (1946) of the stages of development of *Rana pipiens,* based primarily on the differentiation of the limbs, can be used to excellent advantage in comparative taxonomic studies of ranid tadpoles.

In our study, a detailed analysis was made of development from fertilization through metamorphosis of the Gulf coast toad, *Bufo valliceps* Wiegmann. The first objective of the investigation was to establish a set of readily identifiable stages of embryonic and larval development. Each morphologically distinct stage was assigned a number. The second purpose of the investigation was to ascertain which larval characteristics could be employed as taxonomic criteria. The nature of the mouth parts, the pigment patterns, and certain linear dimensions of the larvae were recorded for each stage of development. The results of the two phases of the study are described herein.

[1] "Early development" comprises both the embryonic and larval periods. An embryo is designated here as the prefeeding individual, beginning its existence as a fertilized egg and continuing until it has lost its external gills. Larva and tadpole are considered synonymous terms, each referring to the individual that feeds and passes through a period of metamorphosis before assuming the adult shape.

This investigation emanated from previous studies (Volpe, 1952, 1953, 1955, 1956) in which there arose a need for a simple method of definitively designating the developmental stages of various species of toads. Cursory observations of the early development of *B. valliceps* from southeastern Louisiana were made by the junior author in 1953 and 1954. In the spring of 1955, a systematic analysis of the embryos and tadpoles was carried out by both authors. The study was made possible by a grant from the National Science Foundation (NSF-G1319). The authors are indebted to Mrs. Carolyn Thorne Volpe for preparing the final illustrations.

MATERIALS AND METHODS

Adults of *B. valliceps* were collected during the spring of 1955 from shallow, rain-formed pools at Audubon Park, New Orleans. No difficulty was encountered in inducing males collected during the breeding season to clasp females in the laboratory. The eggs were inseminated by the male (during amplexus) as they emerged from the cloaca of the female.

Five experiments (A–E) were performed with eggs obtained from different mated pairs. Embryos and larvae of four experiments (A–D) were observed for the sole purpose of determining the best method of dividing the continuous process of development into discrete stages. The stages described by Shumway (1940) and Taylor and Kollros (1946) for *Rana pipiens* served as a guide. We were able to delineate 46 stages of development.

The embryos as well as larvae in experiments A–D were reared at room temperature in pond water in 12 by 8 by 2-inch white enamel pans. Six pans were used in each experiment; each pan contained approximately 20 individuals. The larvae were fed boiled spinach. The larvae in each of the pans varied considerably in growth rate, size, and viability. The variability could be attributed principally to effects associated with crowding, e.g., failure of some tadpoles to obtain sufficient food in competition with others, influences of the frequency of collisions or body contacts among the tadpoles, and effects of metabolic waste products that accumulated in the medium. Several investigators (Adolph, 1931; Rugh, 1934; Lynn and Edelman, 1936; Hutchinson, 1939) have emphasized that the available space per tadpole is an important growth factor.

The environmental variables were reduced to a minimum in the fifth experiment (E). The numerical data presented in this paper are based entirely on the results of this experiment. The procedures were as follows: four segments, each consisting of 15 eggs, were cut from an egg string deposited by a single female. Each segment was placed in a finger bowl containing 200 ml. of pond water. The four finger bowls were kept in a "Precision Scientific" constant temperature unit set at 25° C. During the prefeeding period (stages 3–25; figs. 1, 2), the embryos developed at identical rates and were of uniform size. Embryos in the late prefeeding stages (stages 18–25; fig. 2) were measured from the snout to the tip of the tail. Measurements were made to the nearest 0.01 mm. with a Filar micrometer eyepiece inserted into a stereoscopic microscope.

When the larvae commenced feeding (stage 26; fig. 3), 50 of them were distributed individually into 9 by 5 by 2-inch white enamel pans containing 400 ml. of pond water and pieces of boiled spinach. The pans were kept throughout the experiment in two 25° C. temperature units, one housing 27 pans and the other, 23. The pond water was changed daily, the water having been brought to the desired temperature prior to the change. A fresh supply of spinach was also provided at each change period. As the tadpoles approached metamorphosis, the water level in the pans was reduced, and pebbles were added to enable the young toad to emerge from the water.

Thus the larvae were reared under conditions as uniform as possible. Each larva was maintained at the same constant temperature; the water surface area available for each was identical (presumably the dissolved oxygen was the same); each was provided with more food than could be utilized in a day; and the pond water was changed daily to remove the metabolic waste products. Consequently, the variations in growth rate and size could be attributed mainly to genetical differences among the tadpoles.

The larvae were divided into two groups for observational purposes. The first group consisted of the 27 larvae maintained in one of the temperature units. Observations were made at approximately 24-hour intervals. Records were kept of the stage of development, pigment pattern, and linear dimensions of each larva. Three measurements were taken: body length, measured from the tip of the snout to the midpoint of the cloacal opening; tail length, measured from the midpoint of the cloacal opening to the tip of the tail; and body width, measured across the widest part of the body. A fourth dimension, total length, was computed by the addition of the values of body and tail length. The tadpoles were narcotized with "M. S. 222" (Sandoz Chemical Company, New York City) during the brief interval in which they were examined and measured. Staging of the tadpoles and measurements were performed by the senior author; the analysis of the pigment patterns was carried out by the junior author.

The mouth parts were examined in the 23 larvae of the second group. At each observation period, one larva was preserved in 10 per cent formalin and studied. This procedure enabled us to follow the changes in the mouth parts throughout larval development.

STAGES OF DEVELOPMENT

Figures 1–5 illustrate the external features of *B. valliceps* from the fertilized egg through metamorphosis. The age in hours represents the period of time required for 50 per cent or more of the individuals to reach a particular stage. The dimension given for each stage is an average value of the total lengths of the individuals that exhibited the characteristics of the stage at the hour indicated. The variations observed are discussed below.

Stages 1–25 (figs. 1, 2) comprise the embryonic period; stages 26–46 (figs. 3–5) constitute the larval period. The larval stages are based primarily on the development of the hind limb buds and the pigment pattern of the tail.

STAGE 1: The egg at fertilization rotates so that the animal hemisphere is uppermost. The animal hemisphere is brown-black; the vegetal hemisphere is cream-white. The eggs are deposited in a single or double row within a jelly tube. The jelly tube consists of two gelatinous coats, the inner envelope being closely applied to the outer. Measurements of 61 fertilized eggs are as follows: diameter of vitellus, 1.23 mm. $\pm$ 0.05 (mean and standard deviation); diameter of outer envelope, 2.78 mm. $\pm$ 0.16; diameter of inner envelope, 2.48 mm. $\pm$ 0.19. No partition separates the individual eggs. The space between the eggs averages 0.32 mm. $\pm$ 0.11. These measurements compare favorably with those by Livezey and Wright (1947).

STAGE 2: The second polar body is released, as revealed by a round clear area at the animal pole.

STAGE 3: The first cleavage forms two blastomeres.

STAGE 4: The second cleavage forms four blastomeres.

STAGE 5: The third cleavage forms eight blastomeres.

STAGE 6: The fourth cleavage forms 16 blastomeres.

STAGE 7: The cleavage furrows are irregular after the fourth cleavage. "Early cleavage" is designated as 24–64 blastomeres.

STAGE 8: "Mid cleavage" is determined by the relative size of the blastomeres and position of the pigment border.

STAGE 9: "Late Cleavage" is determined by the relative size of the blastomeres and position of the pigment border.

STAGE 10: Involution occurs at the dorsal lip of the blastopore.

STAGE 11: The dorsal lip of the blastopore expands into a semicircle, and involution occurs along the semicircular surface.

STAGE 12: A complete blastopore forms, resulting in a circular yolk plug.

STAGE 13: The embryo flattens dorsally, and the neural plate forms.

STAGE 14: The neural folds form as lateral ridges of the neural groove.

STAGE 15: The embryo rotates slowly inside the egg jelly. The neural folds grow together.

STAGE 16: The neural folds close to form a neural tube. Gill plates are distinct.

STAGE 17: The tail bud develops. Ventral U-shaped suckers are conspicuous beneath the slight stomodael depression. Hatching occurs. An early hatching is characteristic of toad embryos. Embryos of *B. arenarum* hatch at stage 17 or as early as stage 16 (Conte and Sirlin, 1952); those of *B. americanus, B. woodhousei,* and *B. cognatus* are also immobile when they hatch (Bragg, 1940a, 1940b, 1940c).

STAGE 18: Muscular movement begins, which constitutes a simple flexure in response to stimulation. Pronephric ridges, visceral arches, and olfactory pits are recognizable.

STAGE 19: The heart begins to beat. External gill buds are conspicuous.

STAGE 20: Gill circulation begins, as revealed by the movement of corpuscles in the gill filaments.

STAGE 21: The cornea is transparent; the underlying lens is visible as a light spot. The mouth opens, and the suckers begin to disappear.

STAGE 22: Blood corpuscles circulate in the tail fin. The tail epidermis becomes transparent. Melanophores (black chromatophores) appear in the head region and on the dorsal tail musculature.

STAGE 23: The opercular fold covering the gills forms on each side. The number of melanophores increases.

STAGE 24: The operculum closes on the right side. Melanophores extend along the dorsal margin of the tail musculature. Xanthophores (yellow chromatophores) appear at irregular intervals on the most dorsal portion of the tail musculature (represented as clear areas in fig. 2).

STAGE 25: The operculum closes on the left side. The melanophores are confined to the dorsal half of the tail musculature. The series of light areas (containing xanthophores) on the dorsalmost portion of the tail musculature are found throughout later stages.

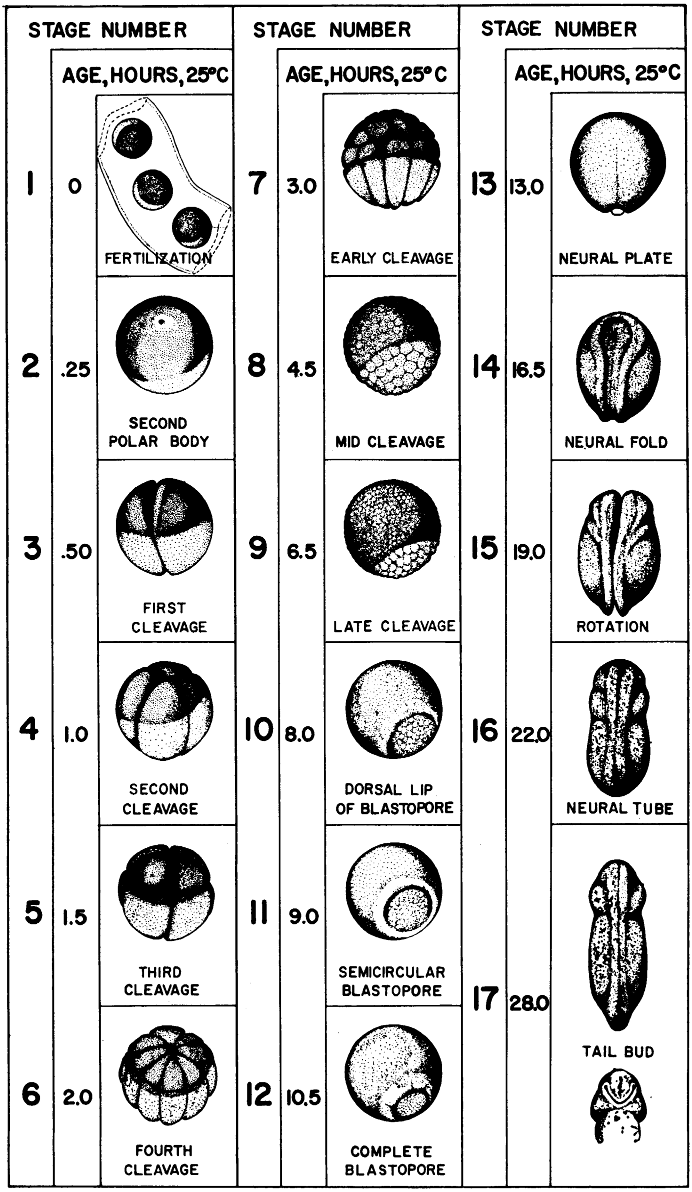

FIG. 1. Embryonic stages 1–17 of *Bufo valliceps*.

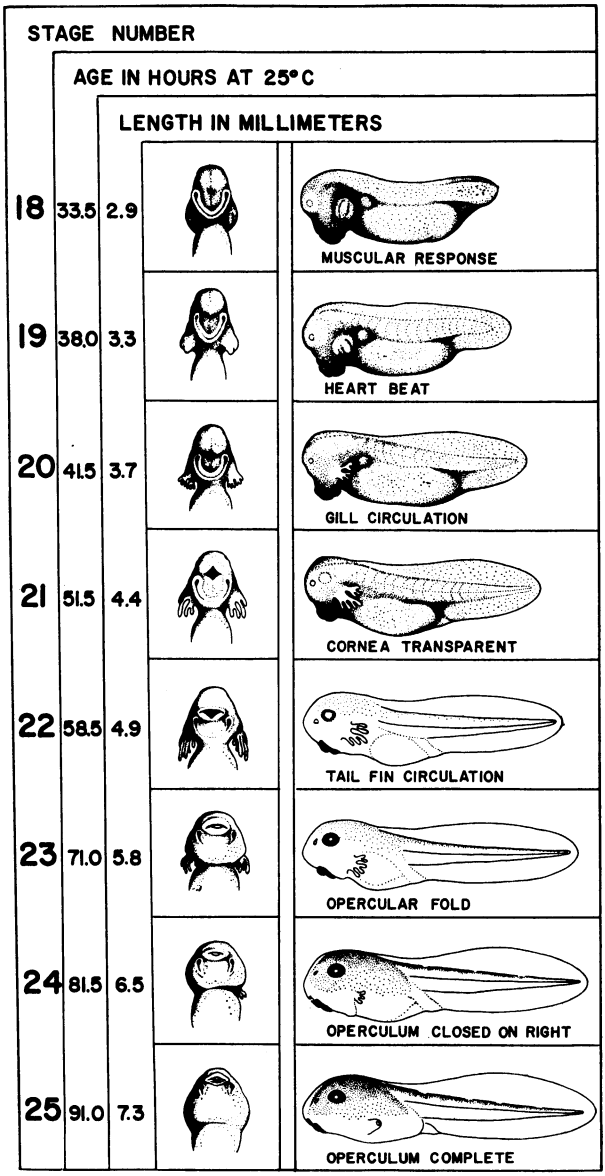

FIG. 2. Embryonic stages 18–25 of *Bufo valliceps*.

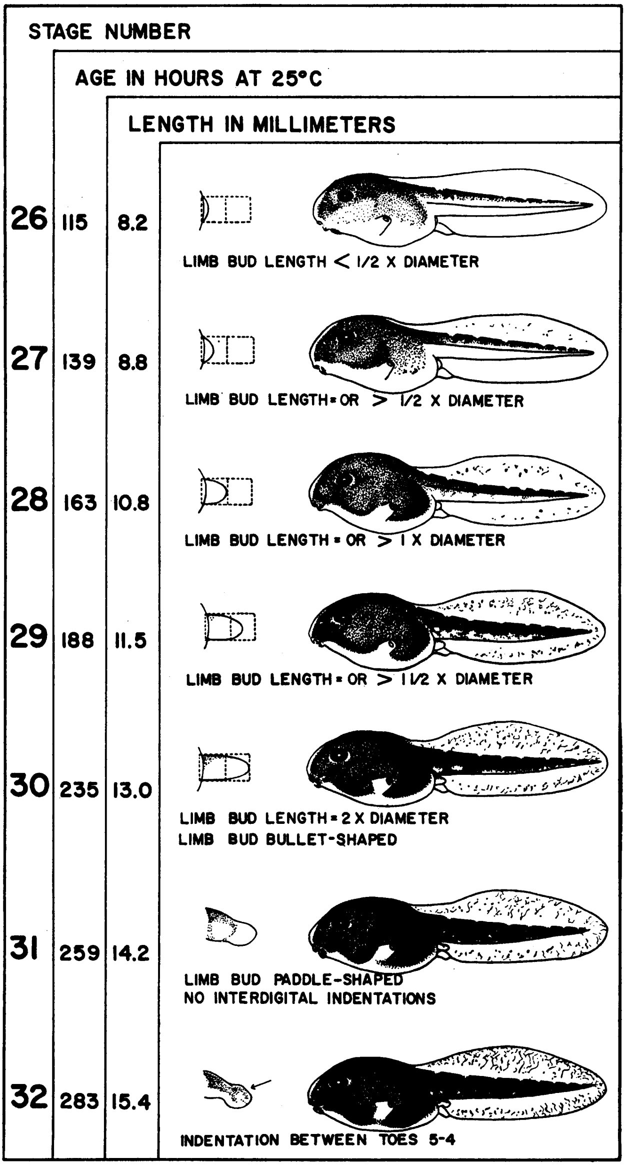

FIG. 3. Larval stages 26–32 of *Bufo valliceps*.

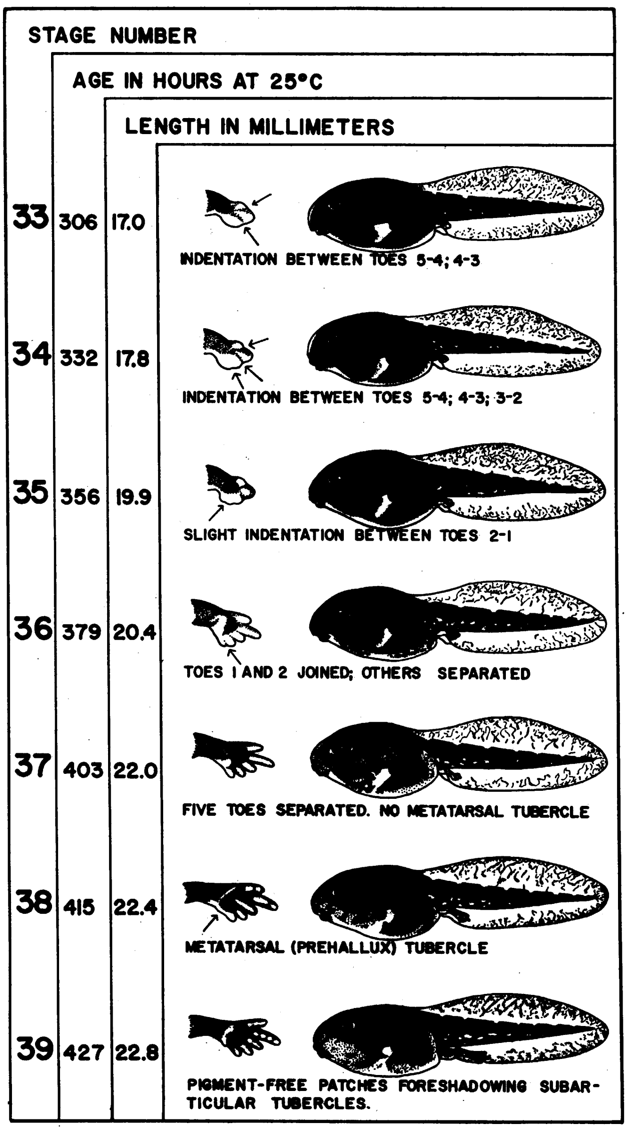

FIG. 4. Larval stages 33–39 of *Bufo valliceps*.

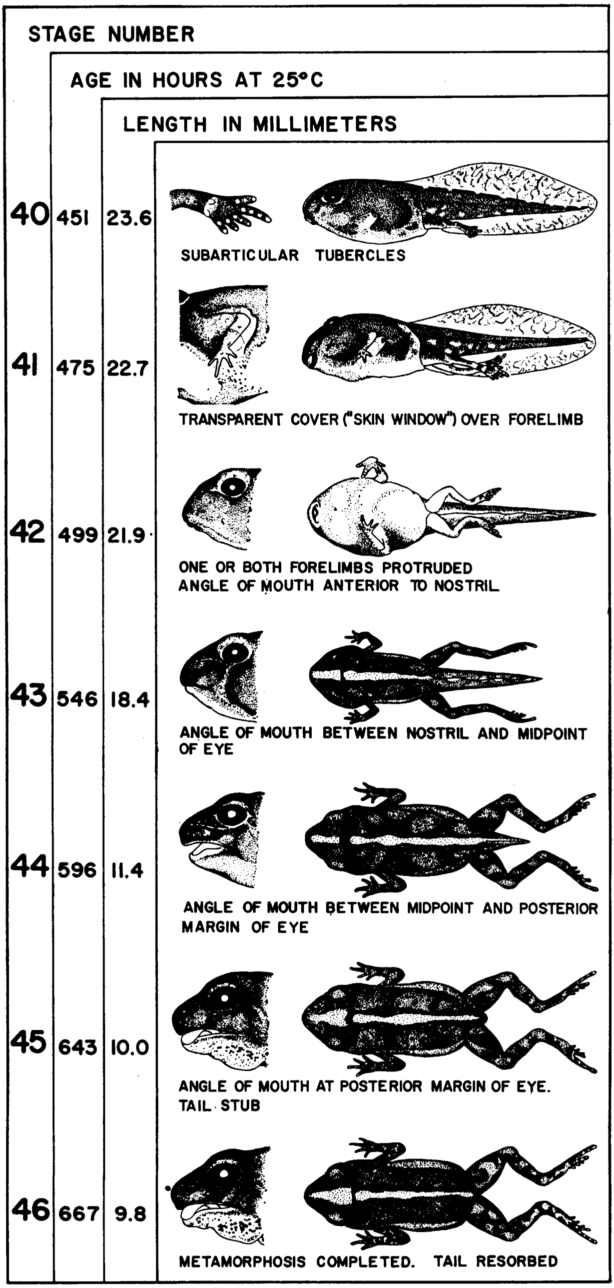

FIG. 5. Larval stages 40–46 of *Bufo valliceps*. The period of metamorphosis comprises stages 42–46.

STAGE 26: The limb bud appears, marking the transition from the embryonic to the larval period. The length of the limb bud is less than one-half of its diameter. The melanophores on the tail musculature progress ventrally but are still confined to the dorsal half of the musculature.

STAGE 27: The limb bud is equal to or greater than one-half of its diameter. Melanophores appear in the dorsal tail fin (membranous portion).

STAGE 28: The limb-bud length is equal to or greater than its diameter. A few melanophores appear on the ventral half of the tail musculature and in the ventral tail fin.

STAGE 29: The limb-bud length is equal to or greater than one and one-half times its diameter. Melanophores are more numerous on the ventral half of the tail musculature and in the tail fins.

STAGE 30: The limb-bud is "bullet-shaped"; its length is equal to two times its diameter. The ventral half of the tail musculature is darkened by melanophores except for several areas or patches which contain xanthophores (represented as clear areas in fig. 3).

STAGE 31: The distal end of the limb bud is paddle-shaped; no interdigital indentations are present on the paddle margin. The number of melanophores increases in the ventral tail fin.

STAGE 32: The margin of the foot paddle is indented between the fourth and fifth toes. The pigment pattern of the tail is characteristic of the remaining stages of development. Melanophores cover the tail musculature except for a few light areas (containing xanthophores) in the most dorsal portion and in the ventral half. The dorsal and ventral fins are heavily mottled by reticulate melanophores.

STAGE 33: The margin of the foot paddle is indented between the third and fourth and the fourth and fifth toes.

STAGE 34: The margin of the foot paddle is indented between the second and third, the third and fourth, and the fourth and fifth toes.

STAGE 35: The margin of the foot paddle is slightly indented between the first and second toes.

STAGE 36: The first and second toes are joined; the others are separated.

STAGE 37: The five toes are separated.

STAGE 38: The metatarsal (prehallux) tubercle appears.

STAGE 39: Pigment-free patches appear on the inner surface of the toes, which foreshadow the differentiation of the subarticular tubercles.

STAGE 40: The subarticular tubercles appear. There are two on the first toe, two on the second, three on the third, four on the fourth, and three on the fifth.

STAGE 41: The skin in the area where the forelimb will protrude becomes thin and transparent. The transparent cover is referred to as the "skin window" by Taylor and Kollros (1946). The cloacal tail piece, i.e., the extension through the ventral tail fin containing the cloacal opening, becomes resorbed. The cloacal tail piece disappears either in this stage or in the following stage.

STAGE 42: One or both forelimbs protrude. The angle of the mouth, as viewed from the side, is anterior to the nostril.

STAGE 43: The angle of the mouth, as viewed from the side, is between the nostril and the midpoint of the eye. The tail begins to regress. Slightly raised warts appear on the back and limbs. A light, median dorsal stripe is bordered in the greater part of its length by a narrow black band, from which branches extend laterally.

STAGE 44: The angle of the mouth, as viewed from the side, is between the midpoint and the posterior margin of the eye. The tail is considerably reduced.

STAGE 45: The angle of the mouth, as viewed from the side, is at the posterior margin of the eye. A slight stub of the tail remains. Most of the lateral black bands on the back disappear. The bands on the limbs begin to anastomose.

STAGE 46: Metamorphosis is completed. The tail is resorbed. The transformed toad has certain conspicuous features of the adult, but lacks others. It exhibits the wide median stripe, the broad lateral stripes, and the irregular pattern of bands on the limbs, but lacks the cranial crests and paratoid glands.

Considerable emphasis has been placed in the above discussion on the distribution of chromatophores in the tail. Individual variations do occur, but the variations are not of sufficient magnitude to obscure the basic pigment pattern. The number of light areas (containing xanthophores) varies from three to 10 in the most dorsal portion of the tail musculature, and may be fewer or greater in the ventral half than the number illustrated in figures 3 to 5. The pattern of melanophores at any particular stage may appear a single stage earlier or later. For example, melanophores first appear in the ventral half of the tail musculature at stage 28; they may arise as early as stage 27 or as late as stage 29. The tadpoles vary also in color intensity throughout development, depending upon the concentration of pigment in the melanophores.

DEVELOPMENT OF THE MOUTH PARTS

Stages in the development of the larval mouth parts are illustrated in figure 6. Complete mouth parts are present only between stages 29 and 40. As illustrated for stage 29, the labial tooth formula is 2/3. In the upper labium, the first upper tooth row is continuous and the second is divided medially. Either lateral segment of the second upper tooth row is approximately twice the length of the median space. The three lower tooth rows are essentially equal in length; the third lower row is slightly shorter than the second, and the second is slightly shorter than the first. Labial papillae are confined to the sides and ex-

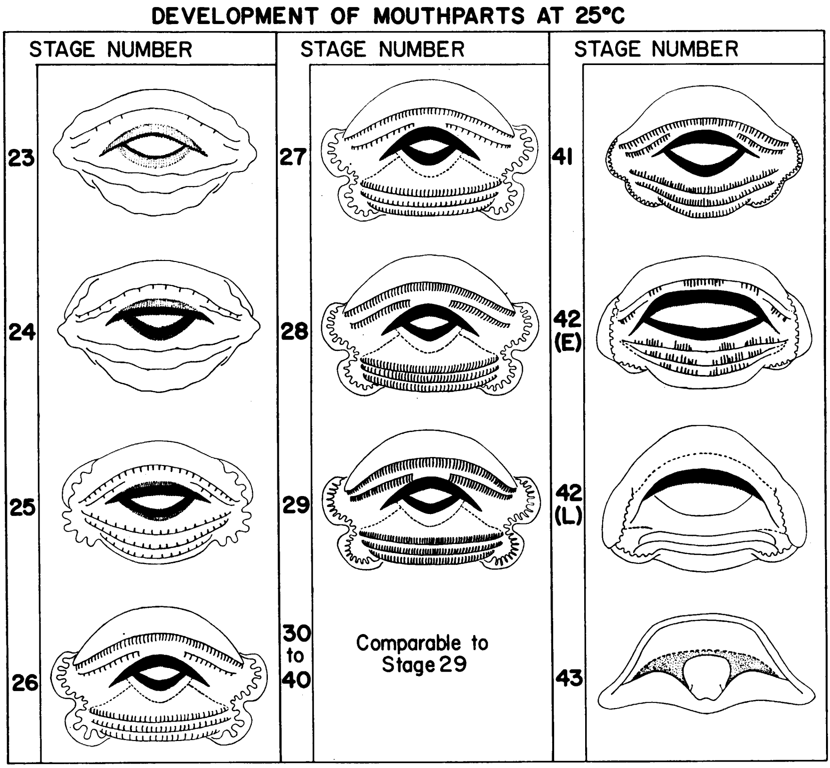

FIG. 6. Stages in the differentiation and resorption of the larval mouth parts of *Bufo valliceps*.

tend to slightly below the edge of the third lower tooth row. Each lateral fringe bearing the papillae is folded inward between the upper and lower tooth rows.

Minor modifications of the above basic pattern may be found in larvae between stages 29 and 40. The first lower tooth row occasionally rises to a point in the midline in the direction of the lower beak. In addition, the first lower row may be interrupted medially by a short space. A few isolated papillae may be present inside the papillary fringes at the ends of the tooth rows.

The mouth parts begin to differentiate at stage 23. Cornified frameworks for the horny beaks appear and become pigmented along the inner margins. A few teeth arise in the first upper row. The other labial ridges do not bear teeth, and marginal papillae are absent. At stage 24, the number of labial teeth increases in the first upper row. More pigment is evident also in the horny beaks. At stage 25, teeth differentiate in the three lower rows and begin to appear in the second upper row; however, no row possesses a full complement of teeth. Marginal papillae are recognizable at this stage. Between stages 26 and 29, the number of labial teeth increases in each row, and the lateral papillae increase in number as well as become progressively smaller in size.

The larval mouth parts undergo resorption at stage 41. The third lower tooth row shortens considerably, and teeth begin to drop out from all rows. The papillary fringes become reduced in size. In an early stage-42 tadpole, a large number of labial teeth is lost, the mouth widens, and the lateral papillae become resorbed. In an advanced stage-42 tadpole, all labial teeth are lost, and one or both of the horny beaks drop out, the lower beak generally before the upper. At stage 43, the mouth possesses structures characteristic of the adult.

ANALYSIS OF GROWTH AT 25° C.

Figure 7 shows the growth curves of total length, tail length, body length, and body width of the tadpoles reared at 25° C. The mean dimensions, expressed in millimeters, are plotted against the time in hours. The hours represented in figure 7 and considered throughout the discussion below refer to the hours that had elapsed since the last of the embryonic stages (stage 25).

The body parts reached their maximum dimensions at 360 hours, at which time the foot of the hind limb was differentiated in the majority of the tadpoles (stage 40). The body parts decreased slightly between 360 and 408 hours when the forelimbs began to emerge. After 408 hours, the shape of the tadpole was markedly altered. The interval between 408 and 576 hours may be considered as the period of metamorphosis (stages 42–46). The variations in body dimensions at

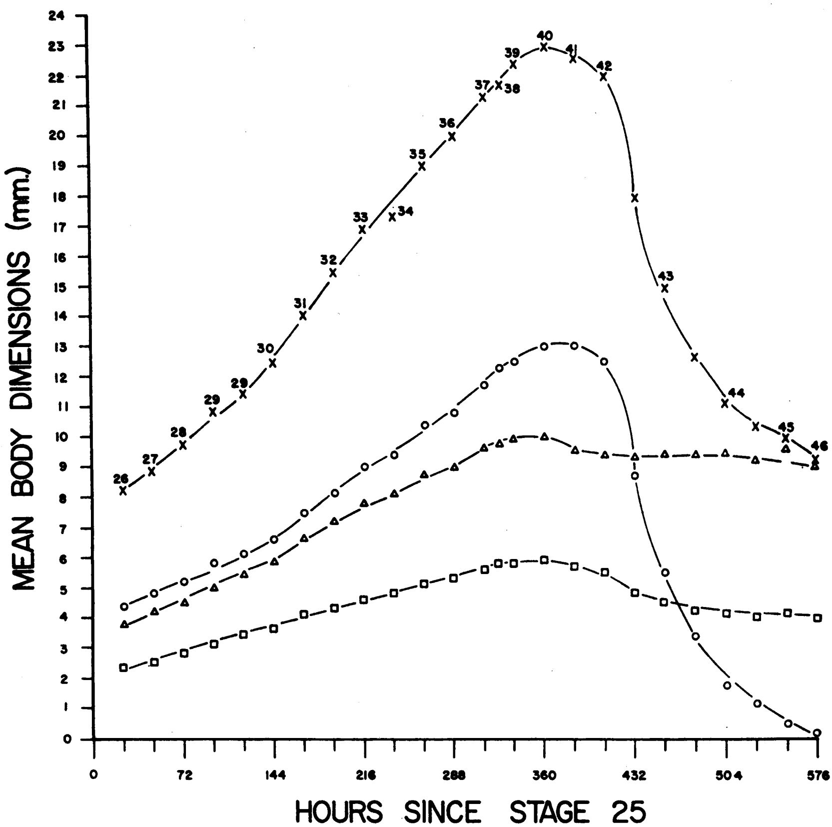

FIG. 7. The average dimensions in millimeters of the total length (represented by crosses), tail length (circles), body length (triangles), and body width (squares) of tadpoles of *Bufo valliceps* at various hours of development at 25° C. The stages of development are indicated at the points of the growth curve for total length and each represents the stage attained by 50 per cent or more of the tadpoles.

each age in hours are indicated in table 1. The mean values shown in the table were used to plot the points in figure 7.

The data of table 1 were employed also in the preparation of figure 8, which illustrates the growth curves of the four body variates as increments in per cent of initial growth. The value of each body dimension at stage 26 was used as the standard of initial growth. To determine the percentage increments of growth of body length, for example, the mean length of the body at each age in hours was divided by the mean body length at stage 26.

TABLE 1

MEASUREMENTS (IN MILLIMETERS) OF TADPOLES OF *Bufo valliceps* REARED AT 25° C.
(Measurements are expressed as mean ± standard deviation and range.)

Hours Since Stage 25	State of 50% or More of the Tadpoles	Total Length	Body Length	Tail Length	Body Width	No. of Tadpoles
24	26	8.2 ± 0.4	3.8 ± 0.2	4.4 ± 0.3	2.3 ± 0.1	27
		7.1 – 8.8	3.3 – 4.2	3.8 – 4.8	2.0 – 2.4	
48	27	8.8 ± 0.6	4.1 ± 0.3	4.7 ± 0.3	2.5 ± 0.2	27
		7.6 – 9.8	3.5 – 4.5	4.0 – 5.3	2.1 – 2.9	
72	28	9.7 ± 0.9	4.5 ± 0.4	5.2 ± 0.4	2.8 ± 0.2	27
		7.9 – 11.5	3.7 – 5.4	4.1 – 6.1	2.3 – 3.3	
97	29	10.8 ± 0.9	5.0 ± 0.4	5.8 ± 0.6	3.1 ± 0.3	27
		8.7 – 12.4	4.2 – 5.8	4.5 – 6.8	2.6 – 3.5	
120	29	11.5 ± 1.2	5.4 ± 0.6	6.1 ± 0.6	3.4 ± 0.3	27
		8.7 – 14.0	4.2 – 6.5	4.5 – 7.5	2.6 – 3.9	
144	30	12.4 ± 1.3	5.8 ± 0.6	6.6 ± 0.8	3.6 ± 0.4	27
		9.0 – 15.1	4.2 – 7.0	4.9 – 7.9	2.6 – 4.3	
168	31	14.1 ± 1.6	6.6 ± 0.7	7.5 ± 0.9	4.0 ± 0.4	27
		9.9 – 16.7	4.7 – 7.7	5.2 – 8.9	2.9 – 4.8	
192	32	15.4 ± 1.7	7.2 ± 0.8	8.2 ± 0.9	4.3 ± 0.4	27
		10.5 – 18.4	5.0 – 8.5	5.5 – 9.8	3.2 – 5.0	
215	33	16.8 ± 1.6	7.8 ± 0.7	9.0 ± 0.9	4.6 ± 0.4	27
		12.2 – 19.6	6.0 – 8.9	6.3 – 10.7	3.6 – 5.3	

TABLE 1—(*Continued*)

Hours Since Stage 25	Stage of 50% or More of the Tadpoles	Total Length	Body Length	Tail Length	Body Width	No. of Tadpoles
241	34	17.5 ± 1.4	8.1 ± 0.6	9.4 ± 0.8	4.8 ± 0.4	26[a]
		13.3 – 19.9	6.3 – 9.1	7.0 – 10.9	3.7 – 5.4	
265	35	19.0 ± 1.5	8.7 ± 0.6	10.3 ± 1.0	5.1 ± 0.3	24[a]
		14.4 – 21.3	6.9 – 9.5	7.6 – 11.4	4.1 – 5.6	
288	36	19.8 ± 1.4	9.0 ± 0.6	10.8 ± 0.8	5.3 ± 0.4	24
		15.4 – 21.6	6.9 – 9.8	8.5 – 12.0	4.1 – 5.8	
312	37	21.3 ± 1.4	9.6 ± 0.7	11.7 ± 0.9	5.6 ± 0.4	24
		16.8 – 23.2	7.5 – 10.4	9.3 – 12.9	4.6 – 6.2	
324	38	21.7 ± 1.4	9.7 ± 0.7	12.0 ± 0.9	5.7 ± 0.4	24
		17.0 – 23.4	7.6 – 10.5	9.4 – 13.2	4.6 – 6.2	
336	39	22.4 ± 1.2	9.9 ± 0.6	12.5 ± 0.9	5.8 ± 0.4	24
		17.4 – 23.7	7.8 – 10.8	9.6 – 14.0	4.6 – 6.3	
360	40	23.0 ± 1.5	10.0 ± 0.7	13.0 ± 1.0	5.9 ± 0.4	24
		18.3 – 24.9	8.3 – 11.9	10.0 – 14.2	4.7 – 6.5	
384	41	22.5 ± 1.4	9.5 ± 0.6	13.0 ± 1.1	5.7 ± 0.5	24
		18.9 – 25.3	8.5 – 10.6	10.4 – 15.0	4.8 – 6.3	
408	42	22.0 ± 2.9	9.5 ± 0.5	12.5 ± 2.6	5.4 ± 0.6	23[a]
		15.0 – 25.4	8.6 – 10.9	5.4 – 16.0	4.1 – 6.3	
432	—	17.9 ± 5.2	9.3 ± 0.4	8.6 ± 4.9	4.8 ± 0.9	23
		9.7 – 25.8	8.5 – 10.3	0.3 – 15.4	3.5 – 7.2	
455	43	14.9 ± 5.7	9.4 ± 0.4	5.5 ± 6.0	4.5 ± 0.7	23
		9.1 – 23.5	8.6 – 10.2	0.2 – 14.4	3.7 – 5.9	

TABLE 1—(*Continued*)

Hours Since Stage 25	Stage of 50% or More of the Tadpoles	Total Length	Body Length	Tail Length	Body Width	No. of Tadpoles
479	—	12.8 ± 4.1	9.4 ± 0.6	3.4 ± 5.0	4.2 ± 0.5	20[a,b]
		9.0 – 23.8	8.7 – 10.8	0.0 – 14.2	3.7 – 5.5	
505	44	11.1 ± 2.8	9.4 ± 0.6	1.7 ± 3.1	4.1 ± 0.3	18[c]
		9.2 – 18.0	9.0 – 10.3	0.0 – 10.2	3.7 – 4.7	
529	—	10.3 ± 1.7	9.2 ± 0.8	1.1 ± 2.3	4.0 ± 0.2	15[d]
		9.2 – 14.2	7.6 – 10.6	0.0 – 6.7	3.8 – 4.4	
552	45	10.0 ± 0.6	9.6 ± 1.1	0.4 ± 0.6	4.1 ± 0.2	10[e]
		9.7 – 10.8	7.7 – 10.8	0.0 – 1.4	3.6 – 4.4	
576	46	9.8 ± 0.8	9.6 ± 0.9	0.2 ± 0.4	3.9 ± 0.2	8[f]
		8.0 – 10.6	7.7 – 10.6	0.0 – 0.8	3.5 – 4.2	

[a] The smaller number is due to the death of one or more tadpoles.
[b] Two of the 20 were in stage 46. These were measured and removed.
[c] Three of the 18 were in stage 46. These were measured and removed.
[d] Five of the 15 were in stage 46. These were measured and removed.
[e] Two of the 10 were in stage 46. These were measured and removed.
[f] Six of the eight were in stage 46. These were measured and the experiment was terminated.

The body grew in length at the same rate as the tail during the early hours of larval development (up to 288 hours or when the majority of the tadpoles were in stage 36). Between 288 hours (stage 36) and 408 hours (stage 42), the tail grew in length at a faster rate than the body. After 408 hours, the body grew in length at a constant rate, whereas the tail decreased in size rapidly. It is of interest to note that the tail grew rapidly just prior to the onset of its resorption. It may be that the increased metabolic activity responsible for the rapid growth of the tail is associated also with its rapid resorption.

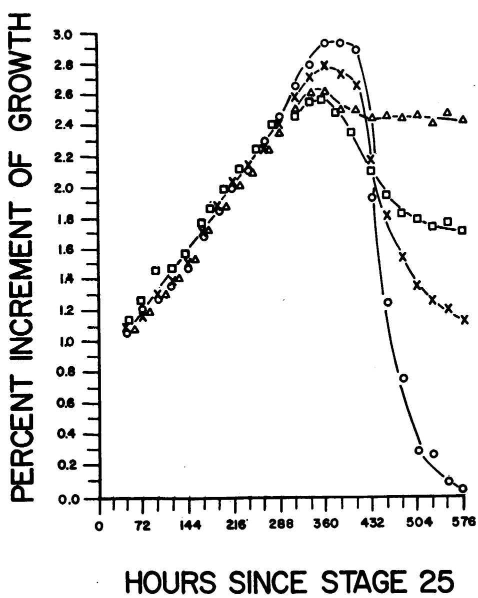

FIG. 8. Curves expressing increments in per cent of initial growth of total length (represented by crosses), tail length (circles), body length (triangles), and body width (squares) of tadpoles of *Bufo valliceps* reared at 25° C.

The body length and body width grew at the same rate up to 384 hours (stage 41). After 384 hours, the body width decreased at a varying negative rate. Thus, in relation to the width, the body became longer as the tadpole approached transformation. The curve for total length represents an interaction of the curves for body and tail lengths.

The four body dimensions are expressed in terms of ratios in figure 9. Mean ratios were calculated from the data of table 1 and plotted against the age in hours. The ratios change little up to 384 hours (stage 41), but, as may be expected, are highly variable during the period of metamorphosis.

The larvae did not attain the same stage of development at the same time, as indicated in figure 10. As many as six stages were found at certain hours of larval development. Consequently, if comparative studies on larvae of other species of toads are to be undertaken, with

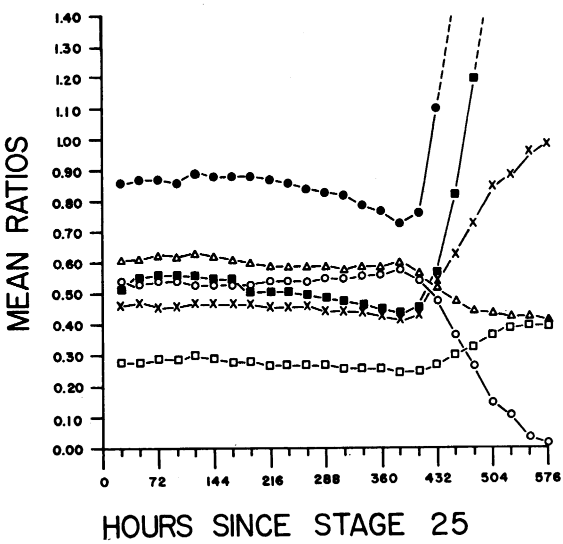

FIG. 9. Mean ratios of body parts of tadpoles of *Bufo valliceps* at different hours of development at 25° C. Body length/total length values are represented by crosses; tail length/total length, empty circles; body width/total length, empty squares; body width/body length, triangles; body length/tail length, solid circles; and body width/tail length, solid squares.

the use of equivalent growth stages, data on mean ratios should be available for each stage of larval development of *B. valliceps*. This information is presented in table 2 and was prepared as follows. At 24 hours (see fig. 10), the 27 larvae, or 100 per cent, were in stage 26. In this case, the mean ratios given in table 2 for larvae at stage 26 are identical to the mean ratios shown at 24 hours in figure 9. At 48 hours, the 27 larvae, or 100 per cent, were in stage 27. However, five larvae, or 19 per cent, were in stage 27 at 72 hours, and one larva, or 4 per cent, was still in stage 27 at 97 hours. Consequently, the measurements of 33 larvae (27 plus five plus one) were used to calculate the ratios presented in table 2 for stage 27. The remainder of the data in table 2 were calculated along similar lines.

TABLE 2

MEAN RATIOS OF BODY PARTS OF *Bufo valliceps* TADPOLES
(Values are expressed as mean ± standard deviation and range.)

Stage	B.L./To.L.	Ta.L./To.L.	B.W./To.L.	B.W./B.L.	B.L./Ta.L.	B.W./Ta.L.	No. of Tadpoles
26	0.46 ± 0.03	0.54 ± 0.01	0.28 ± 0.01	0.61 ± 0.03	0.86 ± 0.04	0.52 ± 0.02	27
	0.35 − 0.49	0.51 − 0.55	0.25 − 0.30	0.54 − 0.66	0.81 − 0.96	0.46 − 0.54	
27	0.47 ± 0.01	0.53 ± 0.01	0.28 ± 0.01	0.60 ± 0.02	0.87 ± 0.04	0.53 ± 0.03	33
	0.45 − 0.49	0.52 − 0.55	0.26 − 0.29	0.55 − 0.66	0.81 − 0.96	0.49 − 0.60	
28	0.47 ± 0.01	0.53 ± 0.01	0.29 ± 0.01	0.62 ± 0.02	0.88 ± 0.03	0.55 ± 0.02	26
	0.45 − 0.48	0.52 − 0.55	0.28 − 0.30	0.60 − 0.66	0.83 − 0.93	0.53 − 0.59	
29	0.46 ± 0.01	0.54 ± 0.02	0.29 ± 0.01	0.63 ± 0.02	0.86 ± 0.04	0.54 ± 0.03	54
	0.45 − 0.48	0.52 − 0.55	0.27 − 0.30	0.61 − 0.66	0.81 − 0.92	0.49 − 0.57	
30	0.46 ± 0.02	0.54 ± 0.02	0.28 ± 0.01	0.61 ± 0.03	0.87 ± 0.04	0.53 ± 0.03	31
	0.45 − 0.49	0.51 − 0.56	0.26 − 0.30	0.58 − 0.64	0.79 − 0.97	0.48 − 0.56	
31	0.46 ± 0.02	0.53 ± 0.02	0.27 ± 0.02	0.60 ± 0.02	0.88 ± 0.03	0.53 ± 0.02	22
	0.41 − 0.49	0.48 − 0.54	0.22 − 0.29	0.58 − 0.62	0.86 − 0.95	0.51 − 0.56	
32	0.46 ± 0.02	0.53 ± 0.01	0.27 ± 0.01	0.59 ± 0.02	0.87 ± 0.06	0.51 ± 0.02	24
	0.44 − 0.48	0.51 − 0.56	0.26 − 0.28	0.58 − 0.62	0.75 − 0.95	0.47 − 0.53	
33	0.46 ± 0.01	0.53 ± 0.01	0.27 ± 0.01	0.59 ± 0.02	0.86 ± 0.04	0.51 ± 0.03	36
	0.43 − 0.48	0.51 − 0.56	0.26 − 0.29	0.55 − 0.63	0.76 − 0.92	0.46 − 0.55	
34	0.46 ± 0.01	0.53 ± 0.01	0.27 ± 0.01	0.59 ± 0.02	0.87 ± 0.03	0.52 ± 0.03	29
	0.44 − 0.48	0.51 − 0.56	0.26 − 0.29	0.56 − 0.62	0.81 − 0.93	0.48 − 0.55	
35	0.45 ± 0.01	0.54 ± 0.01	0.26 ± 0.02	0.58 ± 0.03	0.84 ± 0.05	0.49 ± 0.03	26
	0.43 − 0.47	0.52 − 0.56	0.23 − 0.28	0.55 − 0.62	0.76 − 0.92	0.43 − 0.53	
36	0.45 ± 0.01	0.54 ± 0.01	0.26 ± 0.01	0.59 ± 0.01	0.83 ± 0.04	0.49 ± 0.03	23
	0.43 − 0.47	0.52 − 0.56	0.25 − 0.28	0.57 − 0.61	0.77 − 0.91	0.45 − 0.55	

TABLE 2—(*Continued*)

Stage	B.L./To.L.	Ta.L./To.L.	B.W./To.L.	B.W./B.L.	B.L./Ta.L.	B.W./Ta.L.	No. of Tadpoles
37	0.44 ± 0.01	0.55 ± 0.01	0.25 ± 0.01	0.58 ± 0.02	0.79 ± 0.04	0.46 ± 0.01	23
	0.42 – 0.46	0.53 – 0.57	0.24 – 0.26	0.54 – 0.61	0.74 – 0.85	0.44 – 0.47	
38	0.44 ± 0.01	0.55 ± 0.01	0.25 ± 0.01	0.58 ± 0.02	0.79 ± 0.04	0.46 ± 0.01	20
	0.42 – 0.46	0.53 – 0.57	0.24 – 0.26	0.54 – 0.61	0.74 – 0.85	0.44 – 0.47	
39	0.44 ± 0.01	0.55 ± 0.01	0.25 ± 0.01	0.57 ± 0.03	0.81 ± 0.04	0.47 ± 0.03	19
	0.43 – 0.47	0.54 – 0.57	0.23 – 0.28	0.54 – 0.61	0.77 – 0.91	0.42 – 0.51	
40	0.44 ± 0.01	0.56 ± 0.02	0.26 ± 0.01	0.58 ± 0.02	0.78 ± 0.04	0.47 ± 0.03	30
	0.42 – 0.47	0.54 – 0.57	0.24 – 0.27	0.54 – 0.61	0.73 – 0.84	0.43 – 0.50	
41	0.42 ± 0.42	0.58 ± 0.02	0.25 ± 0.02	0.61 ± 0.03	0.72 ± 0.05	0.44 ± 0.04	34
	0.39 – 0.46	0.53 – 0.60	0.23 – 0.28	0.56 – 0.65	0.66 – 0.85	0.39 – 0.52	
42	0.41 ± 0.03	0.58 ± 0.03	0.23 ± 0.01	0.56 ± 0.04	0.70 ± 0.07	0.39 ± 0.03	27
	0.40 – 0.47	0.53 – 0.60	0.21 – 0.25	0.46 – 0.61	0.67 – 0.89	0.35 – 0.42	
43	0.51 ± 0.07	0.53 ± 0.09	0.26 ± 0.03	0.48 ± 0.03	1.07 ± 1.3	0.51 ± 0.13	24
	0.40 – 0.63	0.36 – 0.59	0.20 – 0.29	0.45 – 0.51	0.66 – 1.36	0.37 – 0.81	
44	0.83 ± 0.09	0.17 ± 0.13	0.35 ± 0.04	0.43 ± 0.02	6.56 ± 4.0	2.80 ± 1.4	31
	0.67 – 0.91	0.08 – 0.32	0.28 – 0.39	0.40 – 0.46	2.11 – 11.41	0.90 – 4.88	
45	0.97 ± 0.02	0.02 ± 0.01	0.40 ± 0.02	0.41 ± 0.02	27.26 ± 12.17	8.49 ± 5.03	19
	0.95 – 0.99	0.01 – 0.04	0.36 – 0.42	0.37 – 0.43	17.10 – 53.90	1.37 –21.90	
46	1.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.02	0.40 ± 0.02	—	—	18
	1.00	0.00	0.38 – 0.43	0.38 – 0.43	—	—	

B.L., body length.
B.W., body width.
To.L., total length.
Ta.L., tail length.

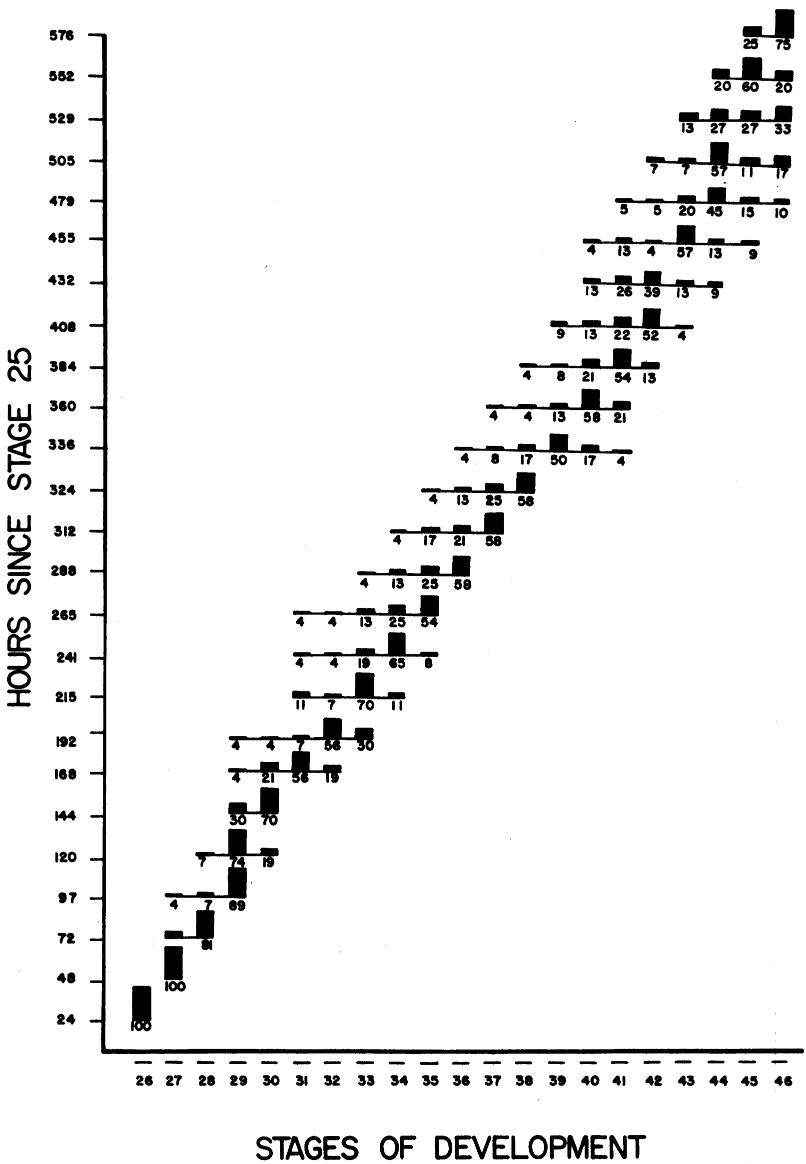

FIG. 10. Bar graph expressing the variations in stages of development of tadpoles of *Bufo valliceps* at different hours of development at 25° C. The number beneath each bar represents the percentage of tadpoles that exhibit the characteristics of the stages at the hour indicated.

An examination of the table reveals that the ratios between any two body components are constant, within the limits of sampling error, up to stage 41 but are highly variable for the metamorphic stages (stages 42–46).

DISCUSSION

Certain characteristics are common to tadpoles of the genus *Bufo* in North America. The generic features are: tooth formula of mouth parts 2/3 (possible exception of *B. boreas exsul* and *B. debilis*); papillae confined to the sides of the labium (may be on lower half only in *B. punctatus*); papillary border on each side emarginate; eyes slightly nearer lateral outline than middorsal line and above lateral axis; spiracle small and on the left side of the body; and a median cloacal opening (Wright and Wright, 1949; Stebbins, 1951; Orton, 1952). These characteristics obviously are of no value in the taxonomic differentiation of species within the genus.

When traits that may be employed as criteria for the identification of species are determined, the choice must be limited to those characters that do not vary, or vary little, during the entire or greater part of larval development. In the past, workers have utilized measurements of various parts of the tadpole, the pigment pattern, and the structure of the mouth parts.

A large number of data on absolute and relative body measurements of tadpoles may be found in the literature. An attempt by us to integrate the data on body measurements has proved futile. Very few systematic studies (e.g., Nichols, 1937; Orton, 1946, 1947) have been undertaken to determine the constancy or inconstancy of absolute or relative body measurements at different growth periods, or to ascertain whether or not there exists a sound basis for the use of body measurements at all in taxonomic work. We have demonstrated that larvae at the same level of development vary in absolute body dimensions and that the dimensions undergo continuous change throughout development. Different growth conditions than those employed by us (e.g., alteration of the quantity and quality of food, temperature, osmotic and pH properties of the growth medium) would undoubtedly result in different absolute body dimensions. Absolute body measurements are too variable to be employed as species differentials.

The absolute body dimensions of *B. valliceps* tadpoles at each growth stage have been transposed into ratios. Relative body proportions are constant during the greater part of larval development (stages 26–41). However, relative body ratios may or may not be of value in the separation of tadpoles of different species. The reliability of relative measurements as taxonomic criteria awaits the accumulation of data derived from comprehensive studies of ontogenetic changes in tadpoles of other species of toads.

The pigment pattern of *B. valliceps* tadpoles appears to be a reliable taxonomic character. Because comparisons with tadpoles of other species should be made with equivalent growth stages, the following description is proposed as diagnostic for *B. valliceps:*

A. Between stages 24 and 41 (figs. 2–5), the dorsal edge of the tail musculature is characterized by an alternating series of light and dark areas. This effect is created by the intervention of groups of xanthophores (yellow chromatophores) among the melanophores (black chromatophores) at regular intervals along the dorsal edge of the tail musculature.

B. Between stages 32 and 41 (figs. 3–5), the larvae are characterized further by a heavy mottling of reticulate melanophores in the dorsal and ventral fins. Moreover, the ventral half of the tail musculature is not darkened completely, but contains a few light areas or patches of yellow chromatophores.

A general indication of the value of the pigment pattern as a species differential may be obtained by a comparison of *B. valliceps* tadpoles with those of other species. It may be mentioned that heretofore relatively little attention has been given to the pigment pattern. The nature and distribution of chromatophores are generally indicated only incidental to other features of the tadpole. Furthermore, the terminology employed is often so vague that an adequate picture of the pigment pattern cannot be formulated. Nevertheless, the following comparisons are offered.

The tail musculatures of tadpoles of *B. americanus* (= *B. terrestris americanus*), *B. terrestris* (= *B. terrestris terrestris*), *B. fowleri* (= *B. woodhousei fowleri*), and *B. woodhousei* (= *B. woodhousei woodhousei*) are covered uniformly with melanophores in the dorsal portion, but are devoid of melanophores in the greater part of the ventral half (Wright, 1914, 1929, 1932; Youngstrom and Smith, 1936; Johnson, 1939; Stebbins, 1951; Orton, 1952; Gosner and Black, 1954; Volpe, 1956). Wright (1914, 1929) described the tail fins of *B. americanus* as "cloudy transparent." Youngstrom and Smith (1936) stated that the tail fins of *B. woodhousei* contain a few scattered flecks of pigment. According to Wright (1929, 1932), the upper tail fin of *B. terrestris* is spotted, but the lower fin is almost clear of spots. The tail fins of *B. fowleri* contain stellate melanophores, more abundant in the dorsal fin than in the ventral fin (Volpe, 1956). It is apparent that the tadpoles of the *B. americanus-B. terrestris-B. woodhousei-B. fowleri* complex have many features in common. More extensive studies of larval development of the members of this species complex should be undertaken. We have observed, for instances, in our study (unpublished) of the larval development of *B. fowleri* that the dorsal fin contains stellate melanophores only during certain growth periods and, further, that the number of melanophores varies with the stage of development.

The muscular portion of the tail of *B. boreas halophilus* is solid black, and the fins are marked with minute melanic stippling (Storer, 1925; Stebbins, 1951; Karlstrom and Livezey, 1955). Stebbins (1951) stated that the tail fins of *B. boreas exsul* are translucent and are stippled with melanophores. Larvae of *B. boreas* (*B. boreas boreas*?) from the Gothic region in Colorado have been described by Burger and Bragg (1947). The tail musculature is densely flecked with dark brown chromatophores, and the semi-transparent fins contain scattered dark brown chromatophores.

The tail musculature of *B. canorus* is dark, as in *B. boreas halophilus*, but, in contrast, the dorsal fin is marked by large, branched melanophores (Stebbins, 1951; Karlstrom and Livezey, 1955). The dorsal tail fin of *B. cognatus* is heavily pigmented with black dendritic chromatophores; the ventral fin is clear or contains a few pigment markings (Bragg, 1936). According to Stebbins (1951), a sparse scattering of minute melanophores in the dorsal fin is characteristic of *B. compactilis*. The tadpole of this species may be readily identified by the series of pigment blotches on the tail musculature that fuse to form an irregular band extending to the tip of the tail (Wright, 1929; Wright and Wright, 1949; Stebbins, 1951).

Smith (1934) described tadpoles, inferred to be those of *B. debilis* (*B. debilis debilis*), from Comanche County, Kansas. The tail musculature is black, and the dorsal fin contains scattered, large, irregularly outlined melanophores. The tadpoles of *B. hemiophrys* bear a striking resemblance to those of *B. americanus*. Stebbins (1951) described the larvae of *B. hemiophrys* (lent to him by Dr. Breckenridge) as follows: ". . . tail musculature colored like body, dark above, lightening slightly ventrally; tail fins translucent, finally stippled with brownish melanophores, more abundant dorsally . . ."

The tail musculature of *B. punctatus* is evenly dotted with black chromatophores (Wright, 1929; Wright and Wright, 1949; Stebbins, 1951). The dorsal tail fin is abundantly supplied with large melanophores (Stebbins, 1951).

The dorsal fin of *B. quercicus* is heavily marked with pigment cells, and the ventral fin is also spotted with melanophores (Wright, 1932). The tail musculature is not uniformly dark but contains several clusters of black chromatophores.

The general impression derived from the review of the tadpoles of the genus *Bufo* in North America is that the pigment pattern in the tail of *B. valliceps* is unique.

The structure of the mouth part may be employed as a taxonomic criterion provided that species comparisons are made with equivalent growth stages. The larval mouth parts of *B. valliceps* are completely differentiated only between stages 29 and 40. During this period, the diagnostic features are the relative lengths of the lower labial rows of teeth and the relative length of the median space in the second upper labial row of teeth. The three lower tooth rows are essentially equal in length; the third lower row is slightly shorter than the second, and the second is slightly shorter than the first. The median space in the second upper tooth row is half or slightly less than half of the length of either lateral segment of teeth. The arrangement of the three lower tooth rows in *B. valliceps* contrasts sharply with the condition found in larvae of *B. americanus, B. fowleri, B. terrestris, B. woodhousei, B. cognatus, B. compactilis,* and *B. hemiophrys*. In the tadpole of each of these species, the third lower tooth row is considerably shorter than either the first or second lower rows (Wright, 1914, 1929, 1932; Bragg, 1936; Youngstrom and Smith, 1936; Nichols, 1937; Smith, 1946; Wright and Wright, 1949; Stebbins, 1951; Orton, 1952; Volpe, 1956).

The three lower tooth rows in the mouth parts of *B. punctatus* are essentially equal in length, but the mouth parts differ in other respects from those of *B. valliceps*. Wright (1929) claimed that papillae are either absent or faintly outlined in the upper half of the lateral margin of the mouth parts of *B. punctatus;* Stebbins's illustration (1951, p. 455) of the mouth parts of this species shows weakly developed papillae in the upper half. Stebbins found also that the second upper tooth row is only slightly shorter than the first and is narrowly divided in the midline.

Reëxamination is needed of the larval mouth parts of *B. debilis*. The descriptions by Smith (1934) and Bragg (1955) are at variance. According to Smith, the labial tooth formula is 2/3 and the second upper row is sometimes divided, but never distinctly. The 2/2 formula described by Bragg for the larval mouth parts of *B. debilis* is unique among toad species. The 1/3 tooth formula of *B. boreas exsul* mouth parts (Stebbins, 1951) is also atypical for the genus *Bufo* in North America.

The mouth parts of *B. boreas halophilus* larvae (Stebbins, 1951), *B. boreas* from the Gothic region in Colorado (Burger and Bragg, 1947), and *B. canorus* (which possess mouth parts similar to those of *B. boreas halophilus,* according to Karlstrom and Livezey, 1955), as far as can be determined from the descriptions, bear a resemblance to those of *B. valliceps.* More detailed studies may reveal significant differences among these forms.

Thus the mouth parts of *B. valliceps* larvae can be distinguished from those of most species of North American toads. However, the importance of a comparison of species at equivalent growth stages cannot be overemphasized. It is to be noted that the third lower row shortens during resorption of the mouth parts (stage 41; fig. 6). The mouth parts of *B. valliceps* larvae at stage 41 may be confused with those of other species of toads in which the third lower row is normally short. Detailed analyses of the development of the mouth parts of all species of toads should be undertaken to permit accurate comparative studies.

SUMMARY

The development of *Bufo valliceps* from the fertilized egg through metamorphosis is described. Forty-six stages of development are defined and illustrated. The diagrams are intended as a reference series for comparative studies of embryos and tadpoles of the genus *Bufo* in North America.

Tadpoles at the same stage of development vary in absolute body dimensions. Moreover, the body dimensions undergo continuous change throughout development. Measurements of tadpoles expressed as absolute values are of limited use as taxonomic criteria. Relative body ratios are constant during the greater part of larval development. The usefulness of relative body proportions at taxonomic criteria awaits the study of ontogenetic changes in tadpoles of other species of toads.

The pigment pattern of the tail and the nature of the mouth parts serve best to characterize the tadpoles of *B. valliceps.* Comparisons with tadpoles of other toad species should be made only with equivalent growth stages.

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