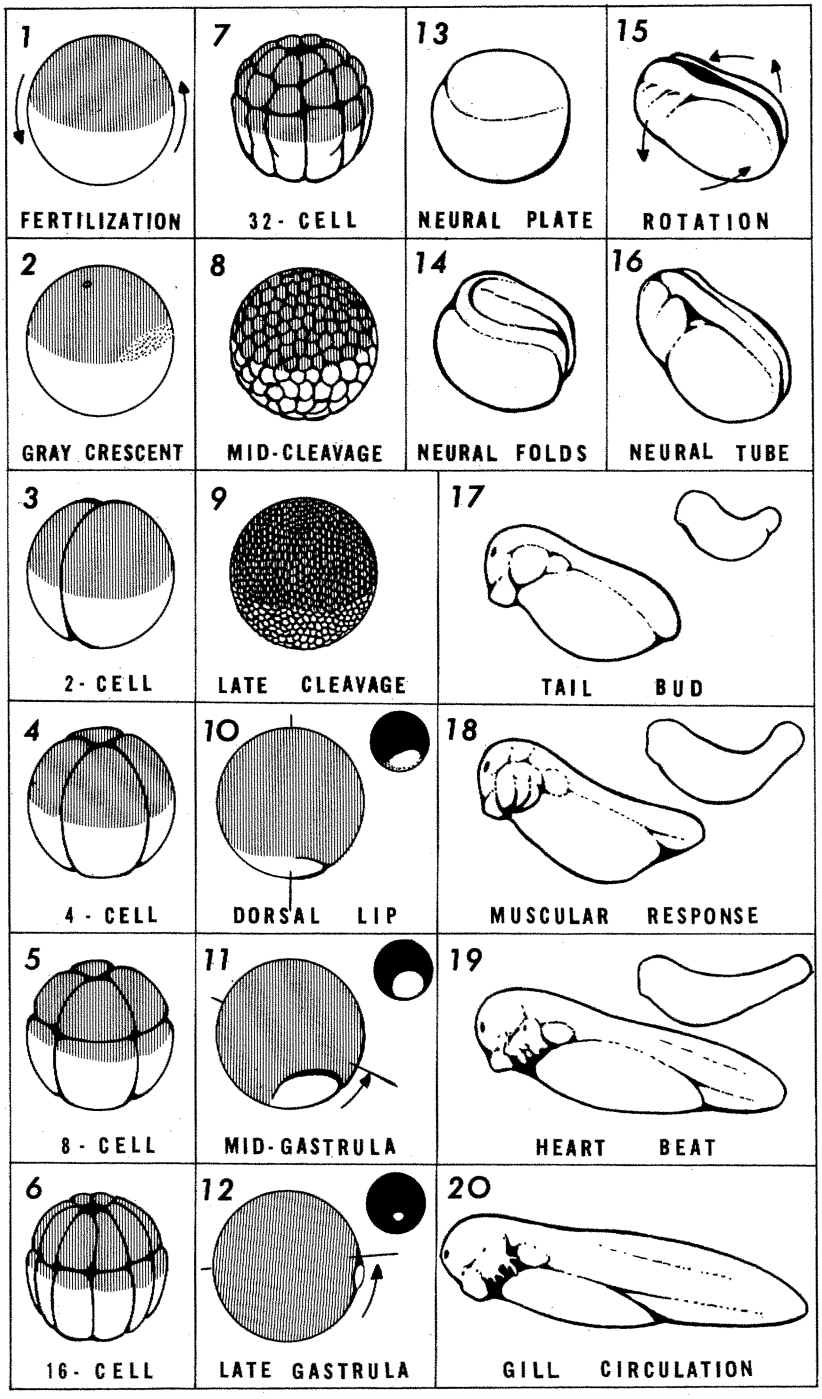
A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification

By KENNETH L. GOSNER

The description of anuran embryos and larvae is facilitated by the use of staging tables, and such tables are indispensible to many studies involving frog life-history materials. Earlier papers in this field by the present author and others adapted existing tables to this purpose. Since this literature now involves two systems for numbering the larval stages, a brief review of the problem is in order, and a simplified table adequate for staging "generalized" developmental series will be presented. The proposed table is original only to the extent that it is a simplification of those already in existence.

Twenty-five prefeeding stages of *Rana pipiens* were tabulated by Shumway (1940), (reprinted in Rugh 1951), and tables, less complete in some cases, are available for several other species (see reviews by Limbaugh and Volpe 1957, and Rugh 1952). The above tables show essentially equivalent stages designated by consecutive Arabic numerals and are adaptable to staging embryos of other species. For postfeeding stages the Taylor and Kollros (1946) table for *Rana pipiens* has proved useful, (also reprinted in Rugh 1951, 1952), and can be adapted for staging other tadpoles. This table shows twenty-five stages designated by Roman numerals. Unfortunately the numeration in the Taylor and Kollros table is not consecutive with that of the embryonic series. More recently Limbaugh and Volpe (*op. cit.*) published a complete table of embryonic and larval stages of *Bufo valliceps* using Arabic numerals throughout and designating forty-six stages. This system was followed in a subsequent paper by Volpe (1959) and also by Gosner and Black (1958), both treating larval toads. The latter authors also indicated that stages in the Limbaugh and Volpe table are essentially equivalent to those of previous authors except that larval stage 40 of Limbaugh and Volpe contains stages XV-XVII of Taylor and Kollros while stage 41 includes stages XVIII and XIX. This reduction in the number of larval stages represents a desirable simplification as, most certainly, is the use of a consecutively numbered sequence for both embryos and larvae. Adoption of this system for general use is, therefore, strongly recommended. To facilitate such adoption the following table is presented, based on that of Limbaugh and Volpe and excluding only details likely to prove specifically variable. Equivalent Taylor and Kollros Roman numeral stages are given for ease of comparison with earlier papers using this system. The proposed table should prove adequate for staging developmental series of most North American pelobatids, bufonids, hylids, and ranids, at least. Since identification of stages is most readily ac-

TABLE 1


1
FERTILIZATION
7
32- CELL
13
NEURAL PLATE
15
ROTATION
2
GRAY CRESCENT
8
MID-CLEAVAGE
14
NEURAL FOLDS
16
NEURAL TUBE
3
2- CELL
9
LATE CLEAVAGE
17
TAIL BUD
4
4 - CELL
10
DORSAL LIP
18
MUSCULAR RESPONSE
5
8 - CELL
11
MID-GASTRULA
19
HEART BEAT
6
16- CELL
12
LATE GASTRULA
20
GILL CIRCULATION

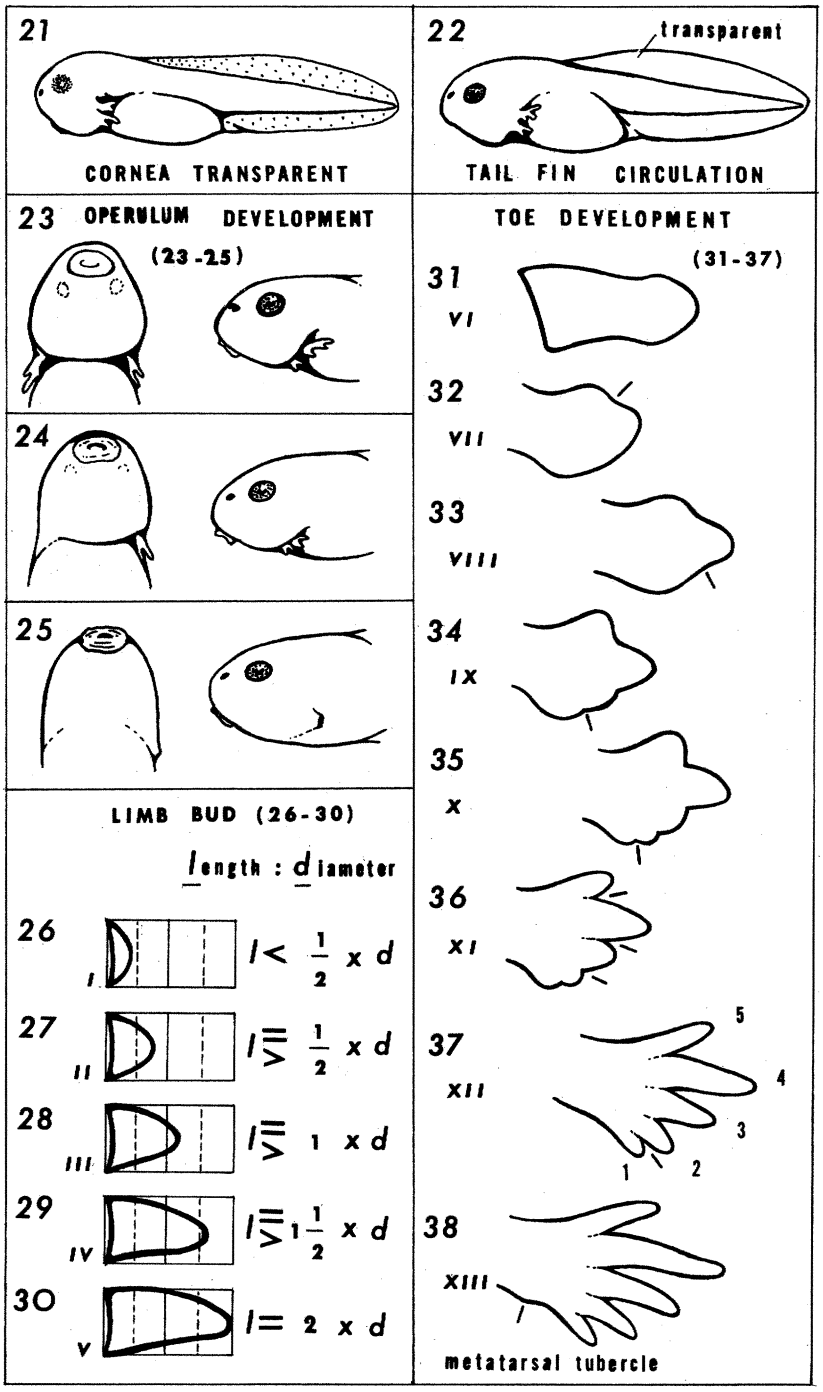
complished by reference to the illustrations, the textual comment will be kept to a minimum. For a full account of early development, see Rugh 1951.

Embryonic Stages—Stages 1 through 25 contain the embryonic or prefeeding series. The number and size of the jelly envelopes and the size and rate of development of the embryo are both individually and specifically variable; hence, such details are excluded from the general table. Egg color also varies systematically among North American anurans. The sequence of changes in the early embryo from fertilization through cleavage, blastula, and gastrula are, however, essentially similar in most species. Fertilization, stage 1, is indicated by rotation of the embryo until the animal pole is uppermost. In stage 2 the second polar body is expelled and a lightening (grey crescent) appears on part of the pigmented hemisphere opposite the point of sperm penetration; these details are not conspicuous without close examination. Seven cleavage stages follow, as illustrated; the early cleavages are regular and more or less symmetrical. After the fourth cleavage, stage 6, cell division is less regular. Stages 7, 8, and 9, are differentiated by the size of the blastomers; also, between stages 8 and 9 the light "hemisphere" is reduced in size through expansion of the darker area.

The involution of cells at a point on the boundary between dark and light hemispheres is taken to indicate the beginning of gastrulation, stage 10. In describing new material, measurements of "egg diameter" should be made prior to this stage, if possible, since the embryo will shortly assume an oval shape; also, the perivitelline capsule absorbs a considerable amount of water in subsequent stages and its diameter cannot be taken as an accurate indication of the initial size of the "vitellus". During the period of blastopore formation, stages 11 and 12, the balance of the live embryo shifts, and the blastopore, initially ventral, becomes the posterior pole of the anterior-posterior axis. The small protruding plug of yolk cells gradually disappears, and the neural plate, stage 13, develops as a tabular area on the dorsal surface. Stage 14, neural fold, is marked by elongation of the embryo and the elevation of two lateral ridges separated by the neural groove. The groove narrows and the folds approach each other as periods of active ciliary rotation of the embryo within its capsule begin, stage 15. At stage 16 the neural folds are closed forming a neural tube; the gill plates become conspicuous, and the embryo begins to develop a recognizable "head."

During the succeeding three stages hylid embryos may appear somewhat dissimilar to those of other families because of their strongly arched form. This difference appears with the development of the tail bud, stage 17. Stages 18, 19, and 20 are differentiated mainly on the basis of relative development of the external gills and tail. Division of the gill plate into ridges (visceral

TABLE 2


21
CORNEA TRANSPARENT
22
transparent
TAIL FIN CIRCULATION
23 OPERULUM DEVELOPMENT
(23-25)
24
25
LIMB BUD (26-30)
length : diameter
26
I
l < 1/2 x d
27
II
l ≧ 1/2 x d
28
III
l ≧ 1 x d
29
IV
l ≧ 1 1/2 x d
30
V
l = 2 x d
TOE DEVELOPMENT
(31-37)
31
VI
32
VII
33
VIII
34
IX
35
X
36
XI
37
XII
1
2
3
4
5
38
XIII
metatarsal tubercle

arches) takes place in stage 18, and in stages 19 and 20 there is progressive development of external gill filaments. These vary to some extent both systematically and individually in size and extent of branching. Full development of the external gills comes between stages 21 and 23. In life, stage 18 is recognized by the initiation of spasmodic muscular responses (simple flexures) and stage 19 by the heart beat, a visible pulsation below and behind the gills, most apparent when the embryo is viewed in profile. In stage 20 gill circulation may be seen as a movement of corpuscles through the external gill filaments. Embryos of most species hatch between stages 17 and 20; the stage at which this normally occurs varies systematically and to some extent individually.

Development of the adhesive organs (oral suckers) may be observed through stages 17-21; their form varies both systematically and ontogenetically. In most larvae the organs are united initially as a crescent shaped ridge which becomes bifid at full development; in *Scaphiopus*, however, the sucker remains U or Y shaped. Following stage 21 these organs rapidly disappear, their scars seldom remaining past stage 26 except in *Scaphiopus*.

Stages 21-25 witness the transition to a feeding and free-swimming tadpole. This is a difficult period for species identification. In stage 21 the cornea become transparent and the eyes are clearly discernable; tail fins are still opaque. In stage 22 the fins become transparent and circulation with them begins. Stages 23, 24, and 25 mark the development of the operculum and consequent disappearance of external gills; these changes may be noted most readily by viewing the embryo in ventral aspect. From stage 25 on, a spiracle is present on the left side of most North American tadpoles except microhylids, where it is ventral and near the anus.

In stages 23-25 there is initial formation of pigmentary patterns, chromatophores of several types appearing at about stage 23-24. At least three types of color cells occur in tadpoles. Melanophores contain a dark, relatively insoluble pigment, presumably melanoid in chemical makeup. Lipophores (xanthophores) contain soluble transparent or translucent pigment, usually yellow, orange, or red; this pigmentation often gives the appearance of a "dispersed color," and recognition of individual chromatophores may be difficult or impossible under ordinary viewing conditions. Iridophores (leucophores or guanophores) contain opaque or milky pigment that is altered on preservation; color bodies of this sort presumably contain guanine and are responsible for irridescent and metallic effects. These colors, like those due to lipophores, are lost on preservation.

The formation of head-body patterns is a complex matter in tadpoles, depending initially on melanophores in the deeper tissues and on visceral elements as well. The intensity of overall color varies, chiefly with the number and state of expansion of the mel-

anophores, and is, to some extent, environmentally controlled. Ontogenetic changes in pattern are due partly to an increase in surface pigmentation masking that in deeper tissues. Recent papers show increasing reliance on tail pigmentation patterns for species identification in toads and hylids particularly. These patterns begin to develop at about stage 24. Pigmentation of the tail fins appears to be less reliable for identification than that of the tail musculature. Considerable ontogenetic change occurs in both fin and musculature patterns in some species.

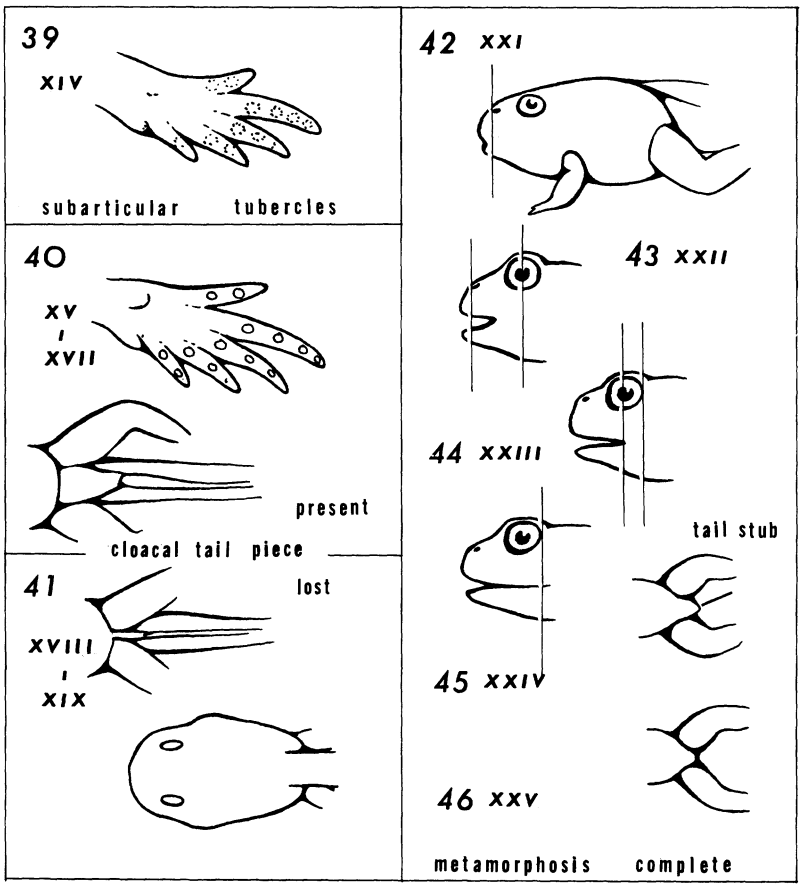
The oral disc and labial tooth rows begin to differentiate at about stage 23. The form of the oral disc is diagnostic for family identification, and its essential pecularities are present by about stage 26, although changes occur subsequently in the number and form of the oral papillae. The tooth rows develop gradually. While the "mature" tooth row formula of a species is usually established in the early larval stages, the relative proportions of the rows change during ontogeny. Allometric change is, perhaps, more pronounced here than in body proportions. The author has followed the custom of examining oral proportions as ratios using the length of the first upper labial tooth row as divisor. In *Scaphiopus* and certain ranids, at least, the number of tooth rows increases during the larval period. There is considerable variation in these traits, and aberrant mouth parts are common in some samples.

Larval Stages—The growth increment between stages 25 and 26 apparently is small in tadpoles of most North American pelobatids, bufonids, and hylids, but amounts to a considerable interval in some of the larger ranids. For this reason the designation of stage 26 as the first larval stage is somewhat arbitrary. It should also be noted that independent feeding actually starts in stage 25.

Identification of stages 26-40 is made by examination of the hind limbs. Stages 26-30 are easily determined by the length/diameter relationship of the developing limb bud. At stage 31 the "foot" is paddle-shaped, and subsequent stages through stage 37 witness the appearance of individual toes. Stages 38-40 are differentiated by proportional changes in the length of individual toes and in the appearance of metatarsal and subarticular tubercles. The latter appear as light patches in stage 39 and as actual tubercles in stage 40.

Limbaugh and Volpe (*op. cit.*) found that ratios of several body proportions in *Bufo valliceps* are relatively constant during this period, i.e. stages 26-40; mouth parts were unchanged between stages 29-40 and pigmentary patterns become stabilized at about stage 32. A comparable study of several species of New Jersey hylid larvae (unpublished data) indicates some allometric change in body part ratios and more extensive changes in labial tooth row proportions (see also Gosner and Black, *op. cit.*). While these changes are relatively slight during a considerable part of the

TABLE 3



larval period, they do complicate the use of such ratios for identification. The extent of change in pigmentation varies in different species. With these reservations in mind we may regard the period between stages 30 and 40, approximately, as one of relative stability in "key" traits.

Following stage 40 the more drastic changes of metamorphosis begin. Total length begins to diminish at this point through resorption of the tail; the larval mouth parts begin to break down. At stage 41 the skin over the forelimbs becomes transparent; the "cloacal tail piece" may disappear at this stage or shortly thereafter.

Stages 42-46 are identified by metamorphosis of the head indicated by changes in the mouth, particularly. Forelimbs appear in stage 42. At stage 46 metamorphosis is essentially complete. Newly

transformed young may or may not resemble the adults sufficiently to permit positive identification.

The chief value of staging tables lies in their use as a shorthand annotation in describing ontogenetic changes and comparing such data for different species. By plotting total length against developmental stages, size-staging graphs are obtained that permit the use of absolute size as a key trait. Without this correlation size data in keys are frequently of little value. In other studies the indication of staging data may enhance the usefulness of published material.

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COUNTING SCALES OF WORM SNAKES.—Counting scales of *Typhlops* and *Leptotyphlops* has always been a chore until I struck upon a method of stitching the specimens on light cardboard. Cut a small hole through which the snake's head may be thrust for determining head scales. Pass a threaded needle about an inch below the hole, upwards, over the snake and down through the cardboard. Continue similarly to about midbody where another hole has been cut for counting scales around midbody. Snake should lie across hole. Continue stitching to near vent where another hole may be cut to facilitate tail counting. Start counting from head, writing with pencil the number of scales at each cross stitch. Make a pencil mark at vent to facilitate counting to end of body and from vent to end of tail.—*Chapman Grant, Rt. 1, Box 80, Escondido, Calif.*