A Table for the Normal Development of *Rana pipiens*[1]

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In 1918 Harrison prepared a series of arbitrary stages in the normal development of *Amblystoma punctatum* which has never been completely published. Drawings of some of the stages have been included in his papers and those of his students, who frequently refer to the series. In fact, in the later papers the drawings are no longer included since the stages have become well known. There are at least forty-six stages in this series, but there is no attempt to correlate the age of the embryo and the stage and no reference to the temperature at which development took place. In 1937 Pollister and Moore published a chart for the normal development of the frog, *Rana sylvatica*. This chart differs from the Amblystoma series by having only 23 stages which are correlated with age, temperature of development, and size in the older stages. These stages are also arbitrary but mark rather easily determined steps in development.

The usefulness of such a series or chart is shown by the frequent reference to the one of Amblystoma. The value of similar charts for anurans used in experimental embryology is threefold. First, the charts would place in the literature standard series of developmental stages for reference. Second, they would simplify the description of experimental results since it would no longer be necessary to include detailed diagrams and descriptions of the stages used. Third, they would reduce the necessity for large control groups since the normal stages are already known. This paper presents such a chart for the normal development of *Rana pipiens*.

Rana pipiens lays in the vicinity of Bloomington, Indiana, from about March 1 until April 15. Most of the eggs used for this study were collected from ponds around Bloomington, brought into the laboratory, and placed in a dark room with a constant temperature of 15° C. The earliest stages were secured from eggs laid in the laboratory and supplied to me from other experiments. These eggs were placed under the same conditions as those collected from the ponds and showed no differences in development.

These eggs for the earliest stages were secured by pituitary injection of the adults, using the method of Rugh ('34). Rugh found that ovulation can be induced in *Rana pipiens* at any season of the year by the injection into the body cavity of whole or macerated frog pituitaries. He found that the most effective method was to inject two female pituitaries daily. This resulted in ovulation on the second to fourth day. If fertilization was desired, amplexus could be induced by pituitary injection of both the male and female, or the eggs could be stripped from the uterus into a sperm suspension prepared from macerated testes.

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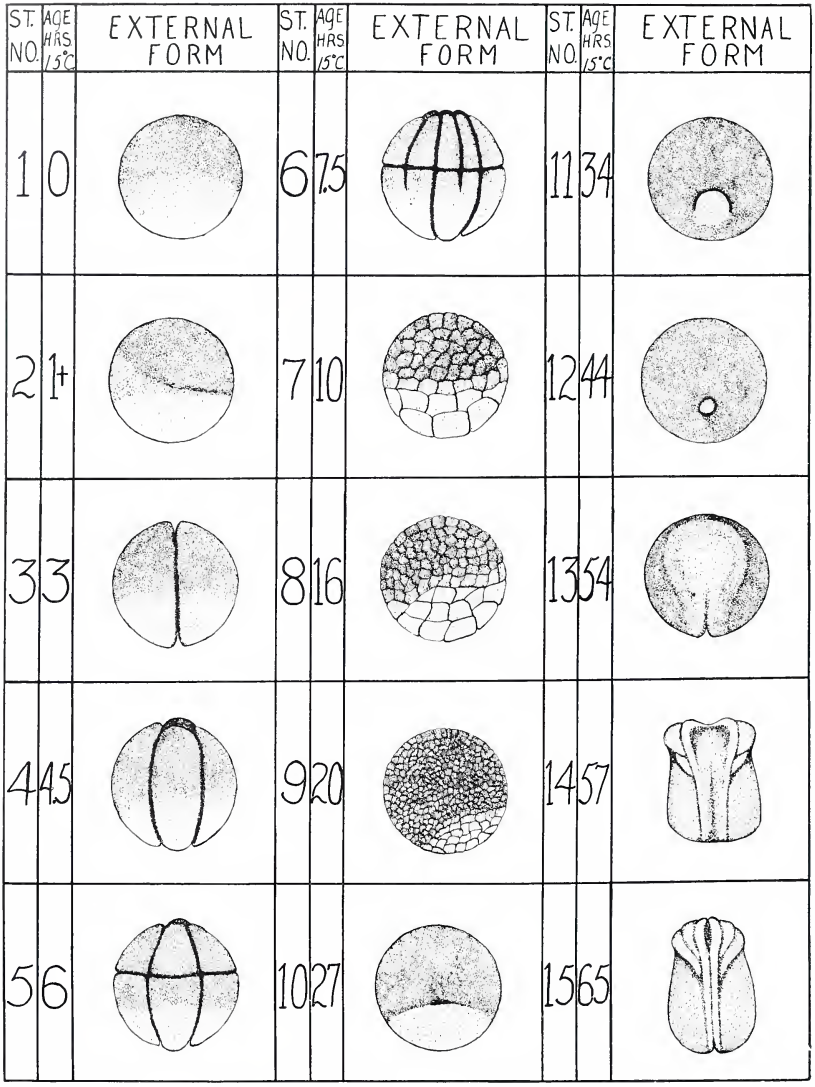
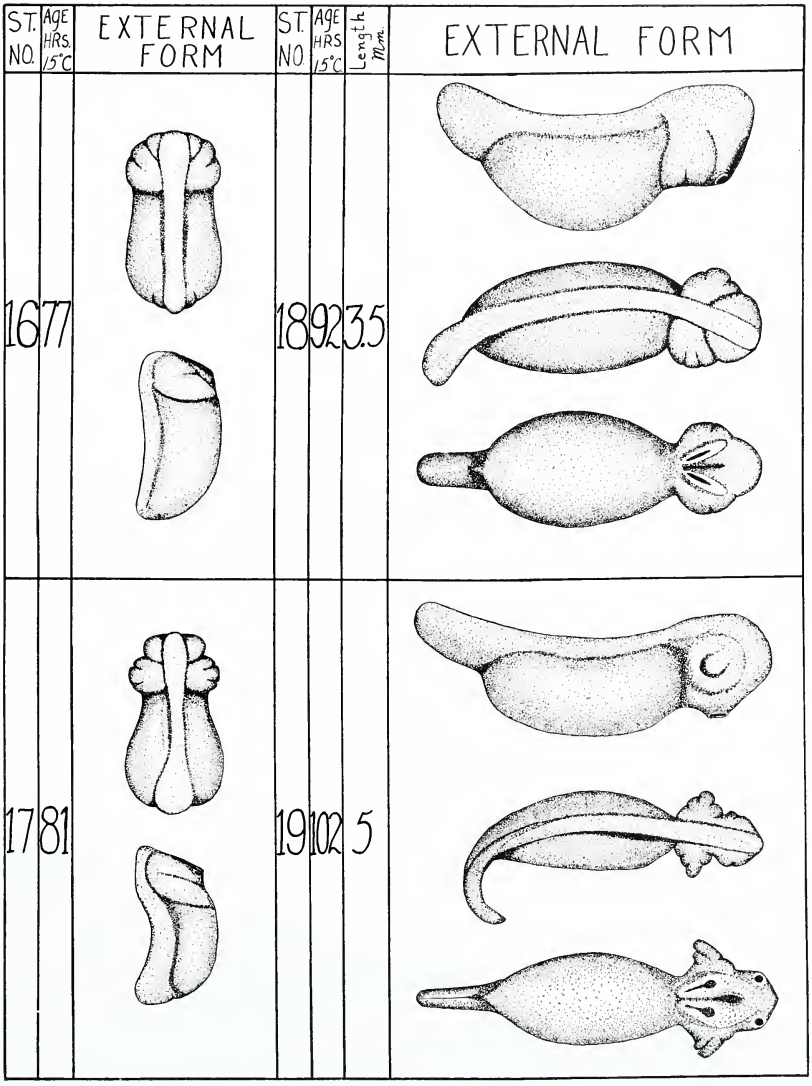
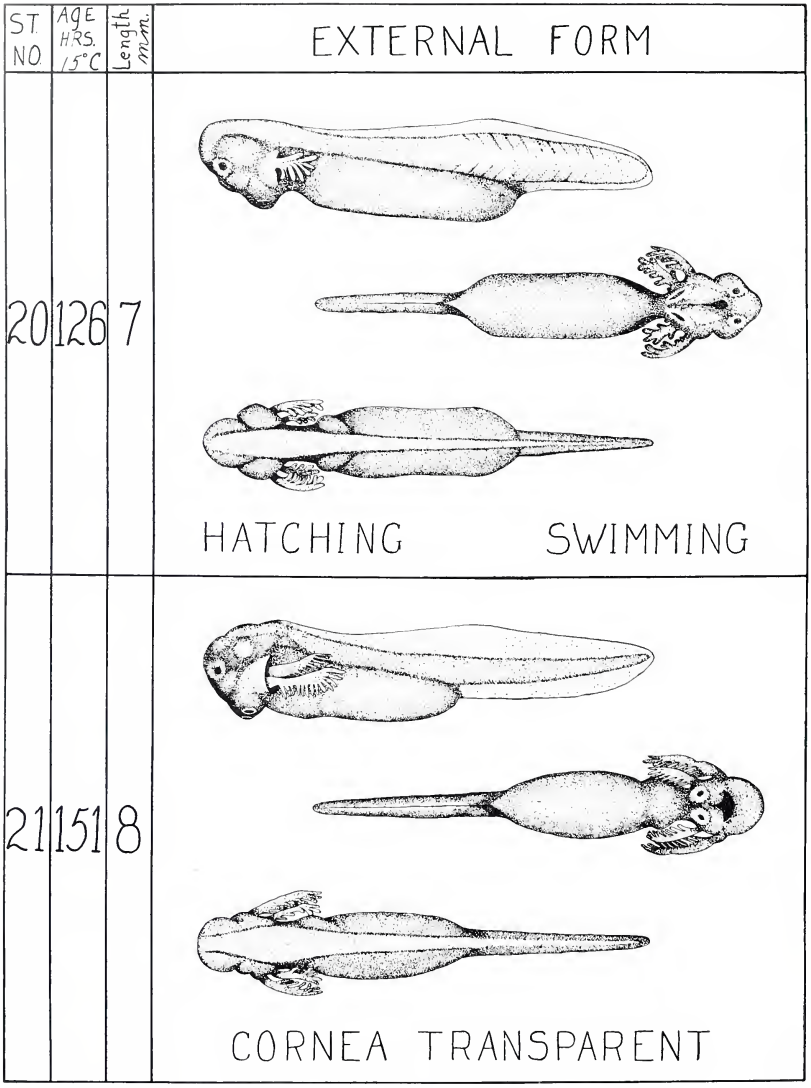


Every two hours, after the 16-celled stage, the developing eggs were studied and sketched from the live specimens. At the same time 20 to 40 eggs were fixed in Tellyesnicky's fluid and preserved in 5% formalin. These preserved eggs verified the sketches and formed the basis for the drawings of the stages selected. In each case the drawings are of individuals average in size and extent of development for the group. The stages figured and described correspond as exactly as possible to the ones figured and described by Pollister and Moore for *Rana*

sylvatica. This has been done for two reasons: to prevent a confusion of stages in the literature and to make a comparison of the species possible. The stages are designated by the external morphology and the age in hours after laying. For the earlier stages the number of cells, relative size of the cells, or development of the neural tube forms an adequate basis for determination. For the later stages, however, several factors, such as the transparency of the cornea and epidermis, elongation of the body, growth of the external gills, and coiling of the gut are helpful in determining the stage designated. Certain physio-





logical features of development, such as the beginning of muscular movement and of swimming and spontaneous hatching, have been used as stage markers.

Description of Stages of *Rana pipiens*

In the following, the numbers refer to figures 1 to 23.

1. The unfertilized egg.
2. Gray crescent formed.

3-6. Early cleavage stages.

7-9. Stages in cell multiplication. The relative size of the animal and the vegetal hemisphere cells form the best criterion for determination of these stages.

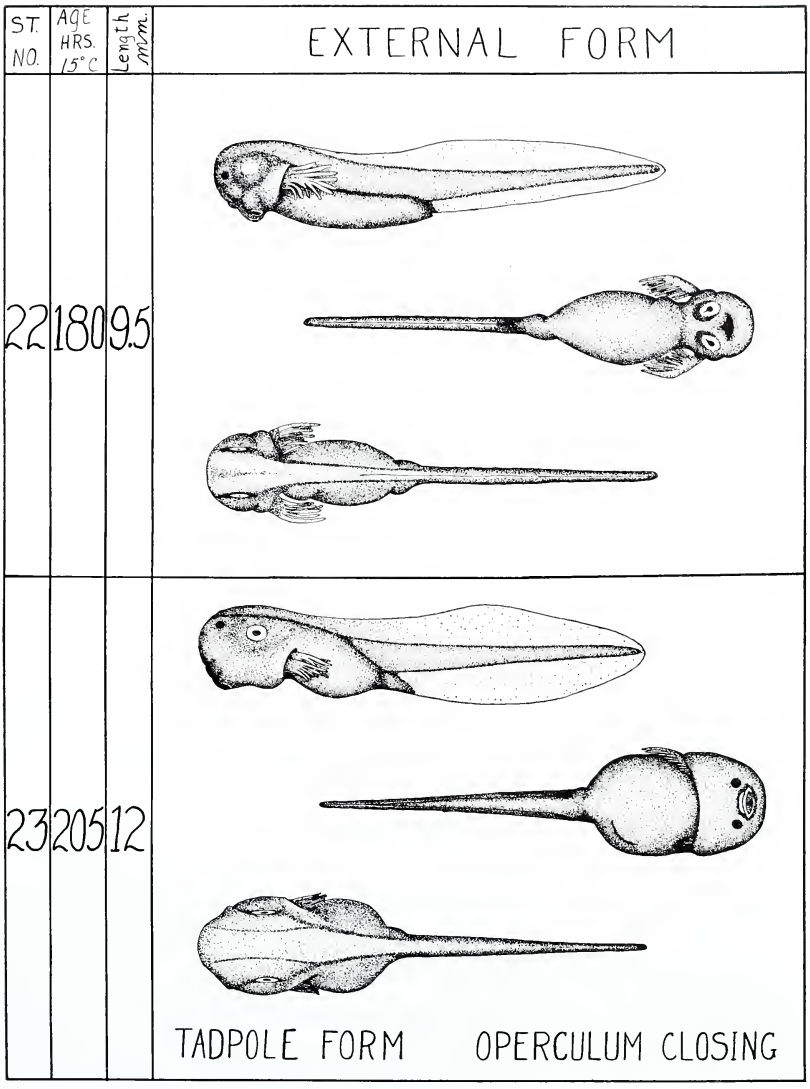
10. Appearance of the dorsal lip of the blastopore.

11. Blastopore almost semi-circular.

12. Blastopore complete, the late yolk plug stage.

13. Neural plate stage.

14. Neural folds formed.



15. Beginning of the closure of the neural tube, also the beginning of the elongation of the embryo.

16. Neural tube completely closed.

17. Beginning of the tail bud. When this stage is viewed laterally, the tail is seen marked off by a notch.

18. Capable of simple lateral movement when stimulated. There is also an indication of the division to form the gills. First distinct curvature of the body.

19. Tail equals one-third of the body length, the division of the gills is becoming definite. The embryo can be hatched by vigorous shaking.

20. Spontaneous hatching occurs in this stage, capable of simple swimming movements when disturbed. The gills are well formed. Tail equals one-half the body length. Epidermis along edges of tail becoming transparent.

21. The cornea is becoming transparent. The fold of the operculum is beginning to form. Tail almost the length of the body.

22. Trunk asymetrical when viewed from dorsal side due to coiling of intestine. Operculum well formed, tail longer than the body. Epidermis of tail transparent.

23. The embryo assumes the tadpole form. It shows active spontaneous swimming. Horny larval teeth are formed. The posterior limb bud is identifiable. The operculum is closing over the gills. Tail three times the length of body. Epidermis transparent so that the gut is visible.

Comparison of the Species

The description of the stages here given is very similar to that given by Pollister and Moore, but this would follow since the stages are made to correspond. However, there are some differences found between *R. pipiens* and *R. sylvatica*. The most noticeable difference is in the gills and operculum. In *R. pipiens* the definite formation of the gills begins about the middle of stage 19. The operculum begins to form in stage 21 and is practically closed by stage 23. In fact, in some embryos of the same age the operculum is completely closed. In *R. sylvatica* the gills begin to form a little later and the operculum is beginning to form in stage 23. In other ways the species are very similar. But however slight the differences may be, this shows further than generalizations for one species cannot be made on the basis of another one even though they are closely related.

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