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Studies of the Early Embryonic Development of *Rana rugulosa* Wiegmann

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Abstract. -This paper deals with the early embryonic developmental stages of *Rana rugulosa* Wiegmann, and with methods of artificial fertilization and experimentally accelerated development. Developmental stages are distinguished by morphological changes and obvious physiological features. At a temperature of 25.1 to 27°C it takes 70 hr 20 min to complete development from fertilized eggs to tadpoles with opercular folds. This course of development is divided into 25 stages, which are standardized with normal table equivalents.

Key Words: Amphibia, Anura, Ranidae, *Rana rugulosa*, embryonic development, artificial fertilization, accelerated development, fertilized eggs, normal table.

Introduction

Rana rugulosa Wiegmann, whose popular name is the Field Chicken in China, is strong and big of body. It is famous for its delicacy of meat and the price is dear. Domestic and foreign markets are in great need of it. The growth and development of these frogs are relatively quick. Hatchlings can become sexually mature in one year. The average body length is about 110 mm. The weight is about 0.25 kg. Consequently, *Rana rugulosa* farming has been a growing enterprise. More papers deal with the embryonic development of *R. guentheri* Boulonger and *R. catesbeiana*. Currently there are few domestic and foreign studies on the embryonic development of *R. rugulosa*. In order to produce reference material for the captive breeding of this frog, we studied its early embryonic development, using artificial fertilization and higher temperatures to accelerate development experimentally, during the months of April and June, 1987 and 1988.

Methods

We made 11 observations on the embryonic development of *Rana rugulosa*. Six of the 11 were artificially fertilized. The parent frogs were bought in Guangzhou. The description of the features of embryonic development was mainly based on the six artificially fertilized eggs. The water temperature of the six developing embryos was 25.1 to 27°C; the pH was 6.5-6.8. Several minutes after oviposition and ejaculation or artificial fertilization, we put the oocyte into the laboratory. Then we used a microscope to observe the course of development and to measure the size and shape. The developmental stages were defined as beginning when half of the embryos in a sample showed all of the distinguishing characters of that stage.

For every developmental stage we took 10 to 20 embryos and fixed them in 5% formalin and Bouin's fluid. This material was used in drawings, tissue-slices, and photomicrographs. The figures shown here were taken from observations of living material.

Results

The 25 stages of the early embryonic of *Rana tigrina* are as follows (time in parentheses is hours and minutes after fertilization):

1) Oocyte stage. Unfertilized eggs are spheres 1.4-1.7 mm in diameter (Fig. 26). (00:25-00:30): The egg membrane absorbs water and expands. The eggs reach 2.6-4.0 mm in perivitellic space. (00:50): The pigment crown of the animal pole extends downward upon the gray crescent (Fig. 1a, 1b, 1c, 1d, 1e, 26).

2) 2 cell stage. (01:12): The first longitudinal cleavage progresses and a cleavage furrow in the animal pole appears, forming 2 equal hemispheres (Fig. 2a, 2b, 27).

3) 4 cell stage. (01:33): A cleavage furrow in the animal pole appears, perpendicular to the first cleavage furrow. It progresses to the vegetal pole and forms four equal sections (Fig. 3a, 3b, 28).

4) 8 cell stage. (01:42): The third cleavage furrow appears parallel to the equator, approximately bisecting the animal hemisphere, forming four small animal cells and four large vegetal cells (Fig. 4a, 4b, 29).

5) 16 cell stage. (01:48): Observed from the animal pole, the fourth cleavage forms eight blastomeres in two circular or elliptical tiers (Fig. 5a, 5b, 30).

6) 32 cell stage. (02:05): The fifth cleavage is horizontal. The animal pole has eight small cells; the vegetal pole has 8 large cells (Fig. 6a, 6b, 31).

7) Early blastula stage. (02:23): The embryo is at the large cell blastula stage, but the cells are still clearly distinguished. The blastocoel begins to appear in the middle part of the embryo near the animal pole (Fig. 7a, 7b, 32).

8) Mid-blastula stage. (02:50): There are many small blastomeres. The blastocoel continues to amplify (Fig. 8a, 8b, 33).

9. Late blastula stage. (03:18): The blastomeres are the color of a red bayberry. The cell boundary becomes indistinct and the blastocoel expands to its maximum (Fig. 9a, 9b, 34)

10) Early gastrula stage. (05:56): The pigmented crown epiboly of the animal cap occurs, extending to cover over 75% of the embryo. The dorsal lip forms. Involution of cells begins (Fig. 10a, 10b, 35).

11) Mid-gastrula stage. (07:00): The dorsal lip continues to amplify. The cells on the side of the lip are drawn into the future archenteron and a semicircle appears (Fig. 11a, 11b, 36).

12) Late gastrula stage. (08:05): The blastopore shrinks and closes gradually. Eventually, the yolk plug is enclosed in the embryo (Fig. 12a, 12b, 12c, 12d, 37)

13) Neural plate stage. (10:30): The blastopore becomes a fissure, and the embryo begins to elongate along the longitudinal axis, becoming pear-shaped (Fig. 13a,.13b, 38).

14) Neural fold stage stage. (11:50): A fold appears on both sides of the neural plate. The neural groove forms the median sinus of the embryo. The front of the plate is bigger, and the neural fold gradually approaches the dorsal axis from both sides. The embryo elongates to 1.9 mm (Fig. 14a, 14b, 39).

15) Cilial movement stage. (12:40): The neural folds are joining. The embryo rotates within the vitelline membrane (Fig. 15a, 15b, 40).

16) Nerve tube stage. (13:45): The neural tube has formed and the gill plate and the cement gland can be seen (Fig. 16a, 16b, 41).

17) Tail bud stage. (14:55): Two outer gill buds protrude on the side of each gill plate. The total length is 3.0-3.6 mm, and the length of the tail bud is 1/10 to 1/7 of this (Fig. 17a, 17b, 17c, 42).

18) Muscle effect stage. (15:55): Muscular response begins in most individuals. The olfactory organ appears, the cement gland is complete. Total length is 3.3-3.8 mm., and the length of the tail bud is 1/7 of this (Fig. 18a, 18b, 18c, 43).

19) Hatching stage. (16:45): The embryo hatches from the egg membrane and two outer gill budlets with 3-5 branches protrude obviously (Fig. 19a, 19b, 19c, 19d, 44).

20) Heart beat stage. (29:25): The heart

begins to move, the eyes protrude , the pair of otoliths can be seen, and the 3rd external gill matures (Fig. 20a, 20b, 20c, 45).

21) Open mouth stage. (31:10): The membrane covering the mouth splits to show the mouth cavity. The alimentary canal is complete and body segments are obvious. The inverted "V" shape myomeres appear on the side of the embryo. Body length is 5.8-6.2 mm. Tail fin length is half of the body length (Fig. 21a, 21b, 21c, 46).

22) Tail fin blood circulatory stage. (32:00): The cement gland begins to degrade, circulation begins in the tail bud, and tadpoles are able to swim in a straight line (Fig. 22a, 22b, 22c, 47).

23) Gill opercular fold stage. (35:55): The opercular fold appears in the base of the external gill and the intestines form a bow (Fig. 23a, 23b, 23c, 48).

24) Right side operculum closed stage. (55:30): The opercular fold stretches toward the right side, the right external gill is covered and forms the right internal gill. The left external gill is exposed. The intestines have 2-3 twists. Total length is 6-9 mm (Fig. 24a, 24b, 24c, 49).

25) Operculum completion stage. (70:20): The external gill in entirely enclosed in the gill cavity, the posterior of which has an exhalent pore to the left side. The yolk has almost been absorbed, and the tadpoles begin feeding. Total length is 6.8-10.5 mm (Fig. 25a, 25b, 25c).

Table 1 shows N & F normal table equivalents (Nieuwkoop and Faber 1967) of our results.

TABLE 1. Normal table equivalents (Nieuwkoop and Faber 1967) of the developmental stages of *Rana rugulosa* as listed in this paper.

N & F stages	Pan & Liang Stages
1	1
2	2
3	3
4	4
5	5
6	6
7	7
8	8
9	9
$10^1/_4$	10
$10^1/_2$	11
11	12A
$11^1/_2$	12B
12	12C
$12^1/_2$	12D
13	13
15	14
17	15
21-22	16
24-25	17
29/30	18
33/34	19A
37/38	19D
39	20
40	21
41	22
42	23
45	24
47	25

Discussion

1. The circumstances of the early embryonic development of *Rana tigrina, R. catesbeiana, R. limnochiris,* and *R. nigromaculata* basically identical, but *R. rugulosa* has some minor differences in developmental timing.

2. During early embryonic development, we raised *Rana rugulosa* in water of about 25.1 to 27.1°C. It takes only 70 hr, 20 min to develop from oocyte to gill cover stage. Under the same conditions, *R. catesbeiana* is 60 to 70 hr slower, and *R. limnochiris* is 80 hr slower. Speed of development is closely related to temperature. At 26 to 28.5°C this developmental period in *R. rugulosa* takes only 64 hr, 30 min.

3. Fifty-five minutes after fertilization, the grey crescent of *Rana rugulosa* appears. This is identical to the timing of *R. catesbeiana*, but different from that of *R. nigromaculata*, in which the grey crescent can barely be seen, or is not visible.

4. The development of *Rana rugulosa* from the eight cell stage to the 16 cell stage takes six min, while the same period takes 59 min in *R. catesbeiana.*

5. In *Rana rugulosa,* external gill develops 3-5 branches during the period of hatching, while in *R. catesbeiana,* the external gill develops 2-3 branches during the tail bud circulatory stage. The third external gill appears sooner in *R. rugulosa* than in *R. catesbeiana* or *R. guentheri.*

6. Eggs laid by *Rana rugulosa* adhere in a pile or on the surface of the water. A reason for low hatching success is that oxygen levels can be low in the center of the egg mass. According to our observations, eggs at the edge of a mass are easy to hatch. If we separate the masses early enough, adequate dispersion can be achieved. However, the operation must be a careful one, or the egg membranes will be broken and the proper development of the embryo can be affected, causing deformity and death.

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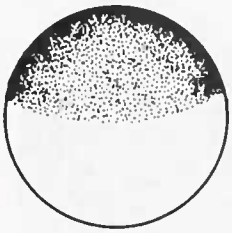


Fig. 1 A

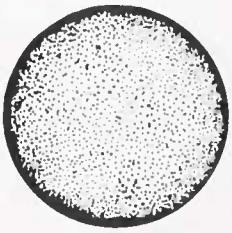
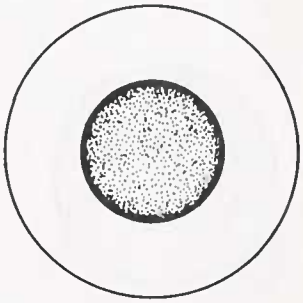


Fig. 1 B



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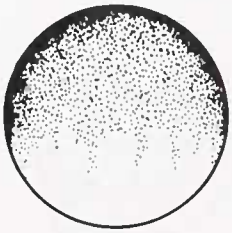

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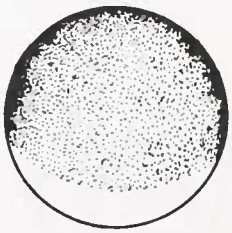
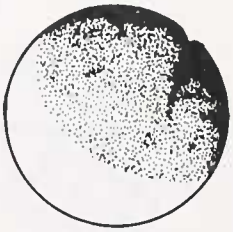


Fig. 1 E



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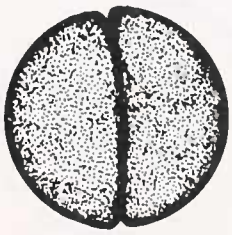

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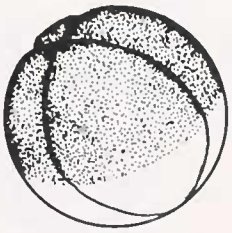

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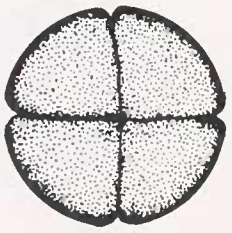
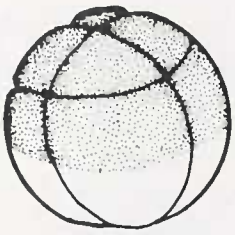


Fig. 3 B

FIG. 1a, 1b, 1c, 1d, 1e, 2a, 2b, 3a, 3b. Embryonic development of *Rana rugulosa*. See text for details.



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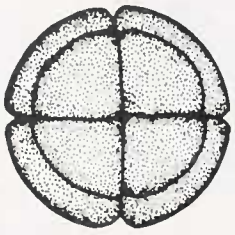
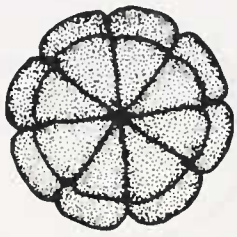


Fig. 4 B



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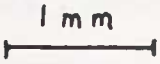


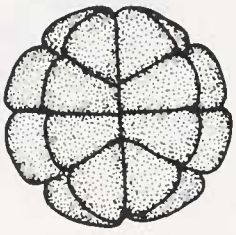
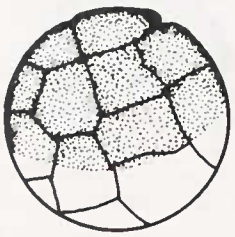


Fig. 5 B



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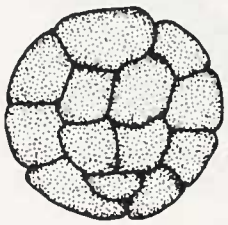


Fig. 6 B

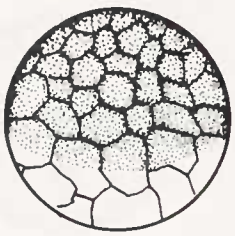
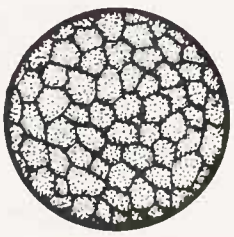


Fig. 7 A



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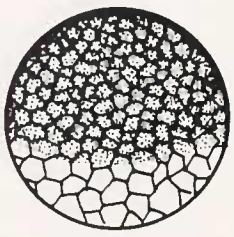


Fig. 8 A

FIG. 4a, 4b, 5a, 5b, 6a, 6b, 7a, 7b, 8a. Embryonic development of *Rana rugulosa*. See text for details.

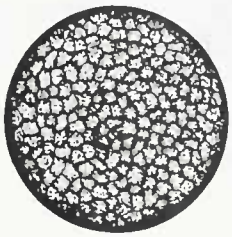


Fig. 8 B

Fig. 9 A

Fig. 9 B

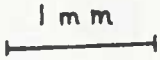


Fig. 10 A

Fig. 10 B

Fig. 11 A

Fig. 11 B

Fig. 12 A

Fig. 12 B

FIG. 8b, 9a, 9b, 10a, 10b, 11a, 11b, 12a, 12b. Embryonic development of *Rana rugulosa*. See text for details.

Fig. 12 C

Fig. 12 D

1 m m

Fig. 13 A

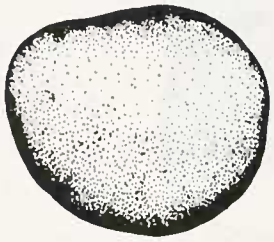
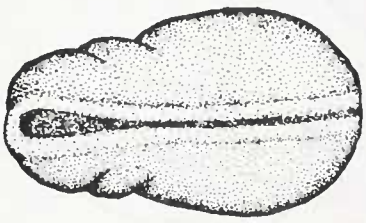


Fig. 13 B

Fig. 14 A

Fig. 14 B

FIG. 12c, 12d, 13a, 13b, 14a, 14b. Embryonic development of *Rana rugulosa*. See text for details.



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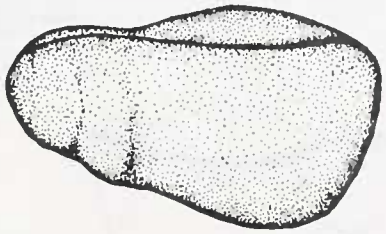

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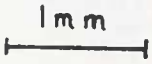


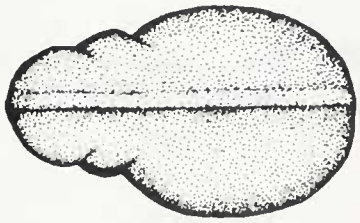
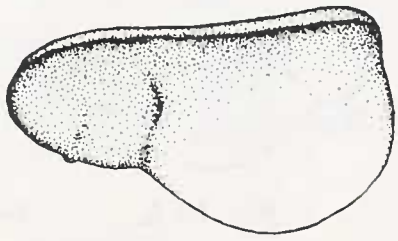


Fig. 16 A



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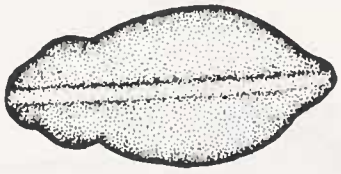


Fig. 17 A

Fig. 17 B

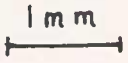


Fig. 17 C

FIG. 15a, 15b, 16a, 16b, 17a, 17b, 17c. Embryonic development of *Rana rugulosa*. See text for details.

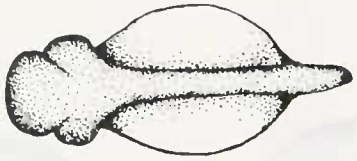
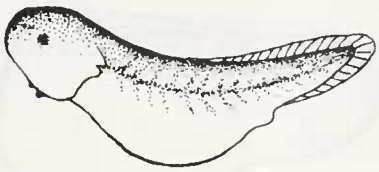


Fig. 18 A

1 m m

Fig. 18 B

Fig. 18 C



2 m m

Fig. 19 A

Fig. 19 B

Fig. 19 C

2 m m

Fig. 19 D

Fig. 20 A

Fig. 20 B

Fig. 20 C

FIG. 18a, 18b, 18c, 19a, 19b, 19c, 19d, 20a, 20b, 20c. Embryonic development of *Rana rugulosa*. See text for details.

Fig. 21 A

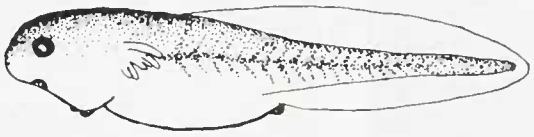


Fig. 21 B

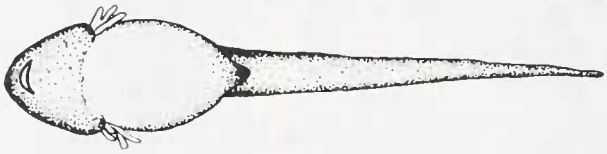


Fig. 21 C

2 mm

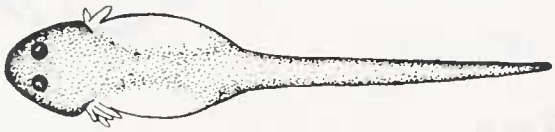


Fig. 22 A

Fig. 22 B

Fig. 22 C

Fig. 23 A

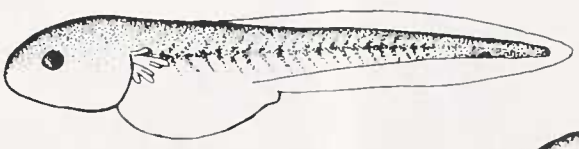


Fig. 23 B

Fig. 23 C

FIG. 21a, 21b, 21c, 22a, 22b, 22c, 23a, 23b, 23c. Embryonic development of *Rana rugulosa*. See text for details.

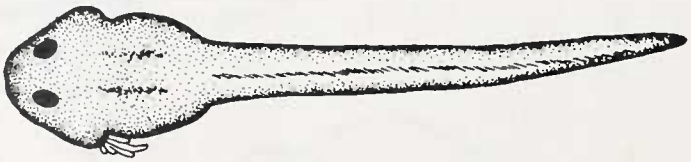


Fig. 24 A

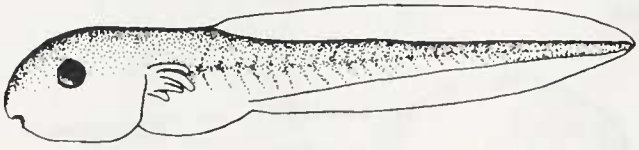


Fig. 24 B

Fig. 24 C

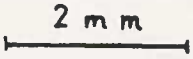


Fig. 25 A

Fig. 25 B

Fig. 25 C

FIG. 24a, 24b, 24c, 25a, 25b, 25c. Embryonic development of *Rana rugulosa*. See text for details.

FIG. 26 (X30)

FIG. 27 (X30)

FIG. 28 (X30)

FIG. 29 (X30)

FIG. 30 (X30)

FIG. 31 (X30)

FIG. 26, 27, 28, 29, 30, 31. Embryonic development of *Rana rugulosa*. See text for details.

FIG. 32 (X30)

FIG. 33 (X30)

FIG. 34 (X30)

FIG. 35 (X30)

FIG. 36 (X30)

FIG. 37 (X30)

FIG. 32, 33, 34, 35, 36, 37. Embryonic development of *Rana rugulosa*. See text for details.

FIG. 38 (X30)

FIG. 39 (X30)

FIG. 40 (X30)

FIG. 41 (X30)

FIG. 42 (X30)

FIG. 43 (X20)

FIG.38, 39, 40, 41, 42, 43. Embryonic development of *Rana rugulosa*. See text for details.

FIG. 44 (X20)

FIG. 45 (X15)

FIG. 46 (X15)

FIG. 47 (X15)

FIG. 48 (X10)

FIG. 49 (X10)

FIG. 44, 45, 46, 47, 48, 49. Embryonic development of *Rana rugulosa*. See text for details.