

The Reproductive Biology of *Rana Boulengeri*

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Abstract.- Maturation of *Rana Boulengeri* Günther occurs at the age of 24 to 36 months. After reproduction the gonads are in an intermittent period. Gonads begin to develop rapidly from next February to April. After May, the ovocytes grow mature in batches. In the wild, the adult frogs often spawn under waterfalls or in shallow pools where the water flows slowly, with high dissolved oxygen. The reproductive period lasts from May to August. The ovulation time of females and fertilization time are generally from 5:00 a.m. to 8:00 a.m. The Water temperature for deposition at the beginning is 15.5°C and the appropriate temperature for most deposition is 17.7-22.5°C. The total duration under artificial constant temperature of 23±0.5°C lasts 261 hours. The appropriate temperature for development is 22-24°C. This species can not develop below 4°C, and it dies at 30°C. The development of the ovary is divided into 6 stages and the development of the testis is divided into 5 stages. The morphological and histological studies at different stages of sex cells are described.

Key words.- Amphibia, *Rana Boulengeri*, reproductive biology, China

Introduction

Rana Boulengeri is a large frog that is found in the Wuling Mountains of Hunan Province. It is an important species for maintaining the ecological balance of the region (Yuan and Wen, 1990). Besides, it can be used as a kind of medicine (Li et al., 1993). In order to help protect this natural resource, these studies on its reproductive biology have been carried out.

Material and Methods

Laboratory experiment and artificial breeding

The studies on the natural condition of habitat, growth and ages, characteristics of reproduction, the development of genital gland and development of the embryo of *Rana Boulengeri* was conducted for four years from 1989 to 1993.

Field investigation

The field sites chosen for investigation were places where *Rana Boulengeri* are common. Field observations were made two or three times a month.

Results and Discussion

Habitat

Natural habitat of *Rana Boulengeri*. The Xiang Xi mountains are a part of the Wuling Mountains, which are located on the border of Hunan, Hubei, Sichuan and Guizhou; between north Latitude 27°44'- 29°48' and east longitude 109°10'-111°20'; with a mean elevation of 686 meters (the highest, 1900 meters above

sea level; the lowest, 75 meters above sea level). The mountains are covered with evergreen broad-leaf sub-tropical forest. The mean air temperatures for a whole year are 16.10 degree-days. The temperature summation above 10°C is 50320; the mean sun hours per year are 1292.7h; frostless period is 270.6 days; the mean rainfall per year is 1397.2mm; the mean humidity per year is above 82% (R.H). These conditions constitute an ideal environment for *Rana Boulengeri*.

Rana Boulengeri frequently live in brooks or ponds in mountain forests where there are few people. The water in the brooks or ponds is very clear. During the day, *R. Boulengeri* often hide at the edge of small caves and during the night, they go out to feed. From July to September of 1987, the physical factors in 35 observation points were measured. Their values are: the elevation of the habitat is 204-675 meters above sea level; air temperature 17.2-24°C; water temperature 14.9-20.5°C; the depth of water 0.2-1.0 meters; the transparency of water 0.2-1.0 m; the humidity 92.0-97.5%.

Activities of *Rana Boulengeri* and temperatures of habitat. Because the air temperature and water temperature play important roles in the development and growth of *R. Boulengeri*; we observed their relationship in a cave (450m above sea level). In autumn, when the water temperature fall lower than 12.5°C, *R. Boulengeri* begins hibernation. In spring, when water temperature is above 12.5°C, they start to feed, and when water temperature is higher than 15.5°C, they enter the reproductive period. The air temperatures and water temperatures in all months are listed in Table 1.

Table 1. Air and water temperatures of the habitat of *Rana Boulengeri* (450m above sea level).

Month	Mean air temperature	Mean water temperature
1	8.1	11.0
2	10.0	12.2
3	10.8	12.8
4	13.0	14.4
5	14.6	14.9
6	16.3	16.1
7	23.9	16.5
8	22.1	16.3
9	18.1	16.1
10	16.1	12.9
11	13.1	11.5
12	8.2	10.0

Chemical properties of the water.

The chemical characteristics of the water inhabited by *R. Boulengeri* were measured and analyzed. The results are listed in Table 2. On the whole, the water is clear, thin, neutral and acidulous, with some contents of Ca, Mg and other minerals.

Vertical distribution. *Rana Boulengeri* are distributed vertically from 200 to 700 meters above sea level, but most of them are found from 450 to 650 meters. Older, large adult frogs are mostly found above 600 meters, while young frogs and tadpoles are found lower.

Growth and age

When bred in artificial pools, the mean body length and the mean body weight of the frogs just completing metamorphosis are 19.8 mm and 0.95 g respectively.

When feeding lasts to the end of the year, their body weight increases to 3.07 g and their body length to 31.2 mm. After feeding for 2 years, their body weight grows to 20.50 g, and the body length to 58.1 mm. The growth curves of body weight and body length are logistic (i.e. "S" shaped) (Li, et al., 1993). In the field, it was found that the largest male was 261 g with a body length of 134 mm. The largest female was 202 g with body length of 120 mm.

Reproductive characteristics

Environmental conditions of breeding areas. In the wild, the adult frogs often spawn under waterfalls or in shallow pools. The mean water area and mean water depth of natural spawn areas are 2.16 m² and 0.35 m respectively. The water flows slowly with high dissolved oxygen. The mean value of pH is 6.3. Generally, there are small stones, sand, ratty plant and leaves, or humus on the bottom. There are bryophytes and algae on the bottom as well as on the sides of the pools.

Reproductive period. In west Hunan at 563 m above sea level, *R. Boulengeri* begin reproduction in May and end in August. The reproductive peak is in June and July. Female frogs may have three clutches, the egg number of the second and third clutches depend on the availability of food and environmental conditions.

Temperature. When water temperature reaches 15.5°C *R. Boulengeri* begins to spawn. The optimum temperatures for large numbers of females to spawn are 17.7-22.5°C. From July to August, the mean air temperature of natural spawning sites is 21.4°C, and the optimum humidity is above 95%.

Reproductive behavior. In middle or late March, *R. Boulengeri* end their hibernation and feed for a month. Then the male and female frogs aggregate at spawning fields. Before mating, male frogs call for about 15 days as a courting period. The ovulation time of females and fertilization times are generally from 5:00 a.m. to 8:00 a.m. When the female spawns, she creeps slowly and her cloaca is near the walls of pool or brooks.

Table 2. Analysis of the water inhabited by *R. Boulengeri* (mg/L). Date of analysis: 5, January, 1990; elevation where water was sampled: 204m above sea level.

pH	total hardness	Ca	Mg	Cu	Zn	Fe	Hg	Mn	Cd
6.71	12.92°C	58.36	13.66	0.013	0.183	0.793	0.001	0.036	0.003
Pb	Ag	Co	Cr	Do	Sulfate	N(NO ₂)	N(HNO ₃)	N(NH ₄)	Water type
0.0148	0.002	0.017	0.0199	4.45	9.89	0.001	0.026	0.075	Thin acidulous

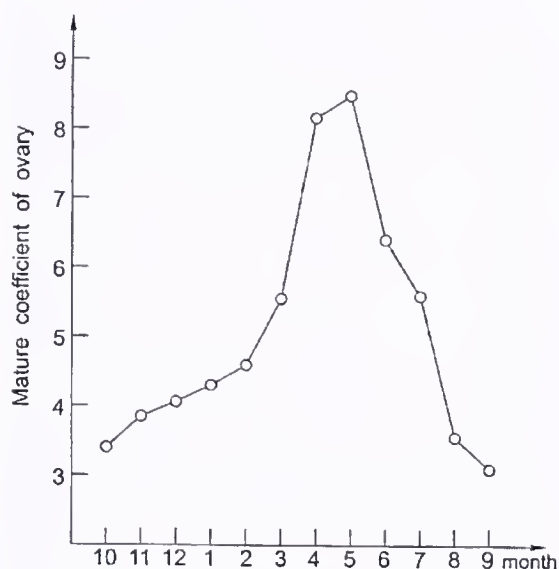


Figure 1. The seasonal change of Mature coefficient of ovary (1991-1993)

Eggs. The mean number of eggs spawned in each batch is about 218. The egg of *R. boulengeri* is big with a mean diameter of 3.98 mm, enveloped by 3 layers of gum membrane (The diameter of egg including membrane is 15.85 mm). The outer layer is the thickest, the inner layer the thinnest. The outer layer is very sticky, so that many eggs connect together to form a long cluster, the end of egg cluster is stuck on the wall or to ratty plants under water.

Development of genital glands

Development of ovary. The development of ovary can be divided into 6 stages.

Stage 1. Multiplication period of ovogonium: The body weights (BW) of young frogs are 7.3-12.5 g and the body lengths (BL) are 37.2-43.6 mm. The ovogonium cells, aggregating densely with the ability of division, have not been surrounded by follicle cells.

Stage 2. Growth of ovocyte (ovocyte): When the BW of young frog are 10.2-19.5 g and BL are 47.2-56.2 mm, ovogonium cells develop into ovocyte cells. The ovocyte clusters are divided by connective tissue of the ovary and surrounded by follicle cells.

Stage 3. Transition growth of ovocyte: When BW of young frog are 36.5-74.2 g and BL are 71.7-84.3 mm, the volume of ovocyte cells increases. The follicle cells increase from 1 to 2 layers and the zona pellucida becomes clear.

Early stage 4. Formation of yolk in ovocyte: When young frogs weigh 58.7-80.8 g and their BL are 81.5-89.7 mm, ovocytes begin rapid growth and particles of yolk begin to form.

Late stage 4. Ovocyte cells filled with yolk: When frogs have BW of 79.7-125.8 g and BL of 87.3-98.0 mm, the ovum cells are fully filled with yolk and the nuclei are isolated in the middle of the cells. The two layers of follicle cells and zona pellucida are well marked.

Stage 5. Slanting of nucleus of ovocyte: When BW are 86.3-161.5 g and BL are 89.4-112.3 mm, the nucleus inclines to the side of animal pole. At this stage, the female can ovulate under the action of gonadotrophic hormone.

Stage 6. Ovary after ovulation: When frogs body weights decrease to 70.6-102.5 g and body length decrease to 82.1-100.2 mm. In this stage, corpora lutea appear in ovary.

Development of testis. The development of testis can be divided into 5 stages.

Stage 1. Multiplication period of spermatogonium: At this stage, spermatogonium aggregates densely, and ranges irregularly.

Stage 2. Formation of sperm-tube: In this stage, the spermatogonium is separated into sperm-tube by connective tissue of testis and part of spermatogonium in sperm-tube becomes spermatocyte of the first order.

Stage 3. Differentiation of spermatocyte: In this stage, the number of spermatogonium in sperm-tube decreases. On the wall of sperm-tube, there are primary sperm mother cells, secondary sperm mother cells and spermatophore formed by spermatoblast respectively.

Stage 4. Formation of sperm: This is the ripe stage of the testis. The spermatogonium and the spermatocyte of the first order are few on the wall of sperm-tube instead of spermatocyte of the secondary order and spermatophore formed by spermatoblast respectively. After the forming of spermatozoon, the spermatozoon aggregates densely first, then disperses.

Stage 5. Post-ejecting: Most spermatozoons have been ejected and only a few could be found in the sperm-tube.

Female order of ovary development and age at sexual maturity. After metamorphosis, the ovaries of the young frogs reach stage 1 in 2-3 months; stage 2 in 6-8 months; stage 3 in 10-13 months; early stage 4 in 21-24 months; late stage 4 and stage 5 in 33-36 months (if ecological conditions are very suitable, in only 22-24 months), when the females can spawn naturally or artificially. After spawning, the ovaries reaches stage 6.

Male order of testis development and age at sexual

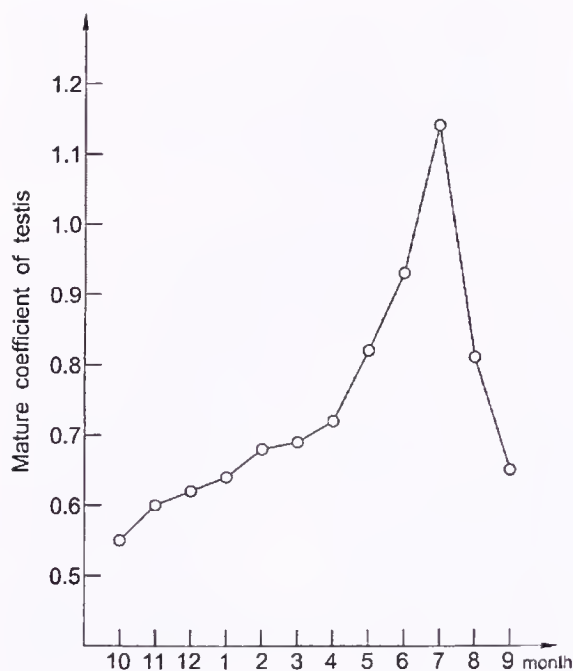


Figure 2. The seasonal change of Mature coefficient of testis (1991-1993)

maturity. After metamorphosis, the testes of males develop into stage 1 in 2-4 months; stage 2 in 8-10 months; stage 3 in 14-18 months; stage 4 in 24-26 months. At this stage the males can mate with the females.

Type of spawning. In the sexually mature females, 3-4 grades of oocyte can be seen. This is the cytological proof of multi-oviposition of *Rana boulengeri*.

Seasonal change of ovaries. The period from May to August is the reproductive time of *R. boulengeri*. Then the ovaries develop slowly and the mature coefficient of ovary (MCO) decreases to 3.10-3.35%. From February to April of the second year, the ovaries develop fast and the MCO reaches its highest value (5.73-14.5%). The mean value of MCO in May is 8.47% (Fig.1). After May, the oocytes mature in batches.

Seasonal change of testes. The testes change little during the different seasons. The range of change of mature coefficient of testis (MCT) in a year is 0.55-1.14%. From April to July, testes develop faster than in other months. In July the MCT is 1.14%. In October the MCT decreases to 0.55% (Fig.2).

Embryonic development

Li et al. (1993) reported in detail on the embryonic development of *R. boulengeri*. Under temperature conditions of $23 \pm 0.5^\circ\text{C}$, 261.05 ± 0.54 hours were required for the embryo to complete its development.

The whole development procedure is divided into 25 stages on the basis of both the morphological and the physiological changes during this period (Pollister and Moore, 1937; Shumway, 1940). The temperature summation above 0°C for the embryo to complete the whole development is 6004.15 degree-hours.

Experiments have shown that temperature can affect the developmental rate of the embryo of *R. boulengeri*. Under conditions of 13°C , 16°C , 22°C , 24°C , 26°C and 28°C , the time for the embryo to complete development is 937.97h, 687.78h, 321.61h, 304.76h, 300.18h and 295.17h. In 2°C and 4°C water the embryo can not develop. In 7°C water the embryo develops very slowly. In 30°C water, the embryo can not complete development. The optimum temperatures for embryonic development are 22°C - 24°C (Li et al., 1994).

Acknowledgments

My research has been supported by the Asiatic Herpetological Research Society. I would like to thank Professor Er-mi Zhao (Chengdu Institute of Biology) for guiding my research and revising my paper. The author wishes to thank Changde Teachers' College for its financial support.

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