

Effect of temperature on the development of early life stages of bull frog

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Abstract: An experiment to investigate the effect of temperature on the development of early life stages of bull frog (*Rana tigrina*, Daudin) for 125 days (May 30 to September 22, 2003) was studied in Aquaculture Departmental laboratory, BAU, Mymensingh. The hatching time and rate varied with different temperature. The highest and lowest hatching time was 93 h at 23°C and 25 hour at 35°C, respectively. Hatching rate was the highest (84%) for 29°C and lowest (65%) for 20°C. The required time for hind limb and fore limbs development decreased with the increment of temperature. At 35°C only 7% tadpoles got hind limb and only 1% got fore limb with abnormal in shape. For the development of hind limbs and fore limbs, 32°C showed the best result. Larval growth was found to be temperature dependent. The highest growth of tadpoles reported at 29°C (53mm) and the lowest at 35°C (32mm). The highest growth showed in 2nd week for 26°C, 29°C and room temperature (26-29°C), and in 1st week for 32°C, 35°C. The best growth performance showed at 29°C. The highest mortality rate observed 90% at 26°C and the lowest at 35°C. It was observed that mortality rate had decreased by rising of temperature. In 20-22°C, all tadpoles died within 6 days of hatching. First completion of larval period (18 days required) at 32°C and was the slowest (29 days) at 26°C. Maximum number of tadpoles at 32°C required minimum number of days (42 days) and at 26°C required maximum number of days (52 days) for completion of their larval period. None of the tadpole completed larval period at 35°C. There was no change in tadpole for the development of limbs at 35°C during the prolonged (69 days) period of the experiment. But it was observed that tadpoles' tails became crooked laterally with a spherical body at 35°C. The larval growth rate and the completion of larval period 32°C showed the best result.

Key words: Bull frog, *Rana tigrina*, Early Life stage

Introduction

Fisheries sector play a vital role in the Bangladesh economy in terms of nutrition, income, employment generation and foreign exchange earnings. This sector consists of piscine as well as non-piscine fishery resources. Non-piscine fisheries composed of shrimp, crab and turtle have contributed significantly (DOF, 2005). Among the non-piscine fisheries resources amphibian fishery is an important one. Now-a-days amphibian fishery mainly consists of frog fishery. Frog legs are a very important exportable commodity of our country. But so far frog fishery is a capture fishery and the frogs (*Rana tigrina*) are exploited from the wild by crude methods at night which take time and labour. Moreover, the frogs are collected mostly during their breeding season irrespective of natural stock. The indiscriminate exploitation of frogs will lead to vanish of the species and consequently bring ecological disaster. In this circumstance, it is urgently necessary to save the frog from being extinction. Frog is one kind of poikilothermic amphibious animal. Frogs make a periodic migration to ponds and streams for the purpose of breeding. Frogs do not only complete egg laying in water but also complete some other process of development of early life stages such as hatching, larval development (limb formation) and so on. This process is called metamorphosis. Therefore, aquatic environment is related with the life cycle of frog thoroughly. Therefore, different physico-chemical factors of water are important for the development of frog. An environmental temperature exerts a direct action in life cycle of bull frog. Especially egg laying, hatching and larval developing stages are very much sensitive to water temperature. Successful reproduction, hatching and larval development depend on temperature mainly. Therefore, temperature has an importance in each stage from egg laying to the completion of metamorphosis of frog. A little work has been done on different aspects of *Rana tigrina* in Bangladesh. Considering the above facts, the present experiment was designed to study the effect of temperature on the development of early life stages of bull

frog under laboratory conditions to find out the effect of different temperature on the larval development, growth, mortality and larval period of *Rana tigrina*.

Materials and Methods

Study area & period: The study was carried out from May 30 to September (125days) in the Laboratory of the Department of Aquaculture, Faculty of Fisheries, BAU, Mymensingh.

Preparation of aquaria: Six glass aquaria (44 cm X 245 cm X 21.5 cm each) and one hatching tray (60.95 cm x 30.48 cm x 15.24 cm) were used for this experiment as the treatment T₁, T₂, T₃, T₄, T₅ and T₆. The aquaria were properly washed and then filled up with over night aerated tap water.

Collection and transfer of fertilized eggs to the laboratory: After heavy rainfall, the eggs which were gelatinous and adhered to grasses and some eggs were settled down on the bottom of the field were collected with the help of a scoop net and transferred to the laboratory. The collected eggs were kept in the previously prepared six different glass aquaria (200 eggs in each glass aquaria). The temperatures of the aquaria were maintained at 20, 22, 26, 29, 32 & 35°C, respectively with the help of thermostat submersible heater. Some eggs were placed in a hatching tray at room temperature (26-28°C).

Study of hatching time and hatching rate: When the larvae comes out it was closely observed the 50% of the eggs hatched and the time taken was considered as hatching time. And when larvae do not come out for a day from the remaining eggs (if any) the relative number of larvae in comparison to incubated eggs was counted and the hatching rate was calculated.

Nursing of newly hatched tadpoles: After hatching just before the yolk sac was completely absorbed the tadpoles were supplied at 5-6% of body weight with fish meal and wheat bran mixture at the ratio of 1:1 as artificial feed twice a day (morning and evening). Water was also changed daily.

Study of the appearance of hind & fore limbs: The time of the first appearance of hind limb of tadpoles and the time of the appearance of hind limb of most of the tadpoles were recorded at each temperature and it was observed carefully with the help of a magnifying glass. Subsequently after the appearance of hind limb the time interval of fore limbs was studied keeping them at 26^o, 29^o, 32^o, 35^oC and room temperature (26-28^oC).

Study of the larval growth rate & mortality rate of tadpoles: In order to study the larval growth rate, 10 tadpoles were caught at random by small scoop net and their individual length was recorded to the nearest mm for each tadpole and for mortality rate of tadpoles, everyday the dead tadpoles within the aquarium were counted starting immediately after hatching. The duration of larval period was recorded by the counting required days from hatching to the complete reduction of tails of tadpoles for each treatment.

Results and Discussion

Hatching time and hatching rate: Hatching time and hatching rate varied with different temperatures. The highest hatching time was 93 h at 20^oC and the lowest was 23^o h at 35^oC. The highest and lowest hatching rate were recorded 84% & 65% at 29^oC and 20^oC, respectively (Fig.1). Chen (1990) reported that hatching time and hatching rate of *Rana tigrina pantherina* (Tiger frog) was 72 h & 90% at 29-31^oC. Das (1995) observed that the hatching time and hatching rate of *Rana tigrina* required 30.93 h and 84% respectively at 26-28^oC. So it is evident that the hatching time was decreasing with the rising of temperature. But at room temperature (26-28^oC) the hatching time was recorded 29 h, whereas, at 26^oC and 29^oC required times were found to be 39 h and 32 h for hatching. At room temperature larvae were in stressed condition due to fluctuation of temperature. To overcome this adverse situation they (larvae) might have increased their activities in the form of movement inside egg and tried to come out from the egg quickly. So in spite of room temperature (26-28^oC) which is lower than 29^oC and higher than 26^oC, it required comparatively shorter time than 26^oC and 29^oC for hatching. Temperature has a direct effect on the hatching time & rate of frog. Bardach *et al.* (1972) noted that the hatching time varied from 3 days to 4 weeks depending on temperature.

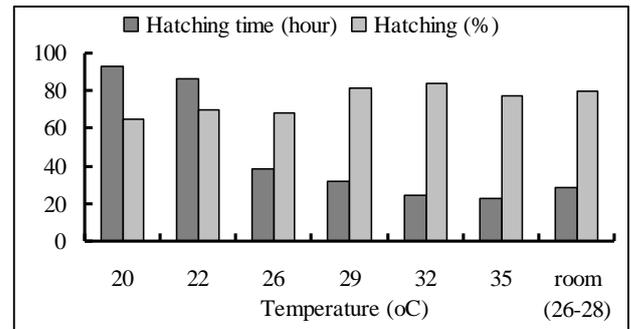


Fig.1. Hatching percentages at different temperature during study

Appearance of hind limb: The hind limb appeared firstly in 15, 10, 9, 16 and 15 days at 26^o, 29^o, 32^o, 35^o and room temperature (26-28^oC). For the appearance of hind limbs of most of the tadpoles were taken 39, 26, 25 and 39 days at 26^o, 29^o, 32^o & room temperature (26-28^oC) respectively. Only 7% tadpoles showed the poorly developed hind limb which indicate that favorable temperature for hind limb development lies some where between 32 and 35^oC.

The longest for appearance hind limb first was at 35^oC whereas; the shortest time was at 32^oC. At room temperature (26-28^oC) this time was same as 26^oC. The formation of hind limb in maximum number of tadpoles at 26^oC was in 39 days which is the same as room temperature. At a glance the information of appearance of fore and hind limbs formation are in Table 1. It is because when the larvae were in stressed condition inside the egg at room temperature, the larvae lost more energy for come out from egg and for match up the lost energy; development occurs slowly even though the temperature has been raised. The critical temperature for hind limb development was found to be around 32^oC. Chen (1976) explained that the time taken for the tadpoles to grow limbs and transformation into young stage varied a great deal according to temperature. Temperature influenced the appearance of hind limb. Among this study of different temperature for appearance of hind limb, 32^oC showed best result.

Table1. Required time for appearance of fore and hind limbs and larval period at different temperature

| Temperature (° C) | Appearance of hind limbs | | Appearance of fore limbs | | Larval period (days) | |
|-------------------|--------------------------|--------------------|--------------------------|--------------------|----------------------|------------------|
| | First appearance | Maximum appearance | First appearance | Maximum appearance | First one complete | Maximum complete |
| 26 | 15 | 39 | 26 | 43 | 29 | 52 |
| 29 | 10 | 26 | 17 | 36 | 24 | 43 |
| 32 | 09 | 25 | 16 | 32 | 18 | 42 |
| 35 | 16 | 50 | 54 | 54 | incomplete | incomplete |
| room (26-28) | 15 | 39 | 20 | 38 | 25 | 51 |

Appearance of fore limb: The first appearance for fore limb was in 26, 17, 16, 54 & 20 days at the respective temperature of 26^o, 29^o, 32^o, 35^oC and room temperature (26-28^oC). Most of the tadpoles have got fore limbs in 43, 36, 32, and 38 days at the temperature 26^o, 29^o, 32^o, and

room temperature (26-28^oC), respectively. Only one tadpole has got fore limb in 54 days at 35^oC temp. But the tadpole was abnormal in appearance. Chen (1976) found that the time taken for the tadpoles to grow limbs and transformation into young stage differs a great deal

according to temperature. Das (1995) noted that the fore limb of *Rana tigrina* formed 35-47 days after hatching at room temperature (26-29°C) which agrees with the findings of this experiment also It was found that at 26°C the time required for the first appearance of fore limb was 26 days . The required time for the first appearance of fore limb was rapidly decreased with increasing temperature up to 32°C. It decreased very rapidly up to 29°C. From 29°C to 32°C the required time decreased very slowly and then suddenly it was very rapidly increased up to 35°C. This change from decreasing to increasing around 32°C indicates the critical temperature for fore limbs

development. . None of the tadpoles except one had developed fore limb at 35°C. From the result obtained it is evident that from the selected gradient the temperature of 32°C gave the best result in terms of limbs development.

Larval growth in rearing period from immediately after hatching up to 4th week: Larval growth was found to be temperature dependent. At different temperatures the highest growth was recorded in different times. The highest growth was in 2nd week, for 26°, 29°, and room temperature (26-28°C) and in 1st week for 32°C and 35°C. The information of growth rate upto fourth week is presented in Table 2.

Table 2: Growth rate of tadpoles from hatching to 4th weeks at different temperature

| Temperature (°C) | Average initial length (mm) | Average length (mm) | | | | | Total growth (mm) |
|------------------|-----------------------------|---------------------|----------|----------|----------|------|-------------------|
| | | 1st week | 2nd week | 3rd week | 4th week | | |
| 26 | 6.5 | 15.4 | 25.0 | 26.1 | 35.0 | 28.9 | |
| 29 | 6.5 | 21.0 | 26.0 | 46.3 | 53.5 | 47.0 | |
| 32 | 6.5 | 24.0 | 25.7 | 34.6 | 45.7 | 39.2 | |
| 35 | 6.5 | 24.2 | 26.0 | 29.4 | 32.0 | 25.2 | |
| room (26-28) | 6.5 | 14.0 | 22.7 | 28.8 | 33.0 | 26.5 | |

Das (1995) reported that the highest growth rate of tadpoles at the first phase of metamorphosis was in the second week at laboratory condition for *Rana tigrina*. The growth rate of tadpoles was found to be influenced by the temperature. Bruneau and Magnin (1980) reported that the high growth of tadpoles of *Rana catesbeiana* occurred mostly in, June July and August and it was varied with temperature. From this study it is also found that 32°C is the best suited temperature among the tested temperatures.

Mortality rate of tadpoles at different temperatures: All tadpoles died 6 days after hatching at 20°C and 22°C. Mortality rate was recorded highest 90% at 26°C followed by 85%, 75%, 70%, and 40% at room temperature (26-28°C), 29°C, 32°C and 35°C, respectively. At 35°C mortality rate was the lowest (40%) (Fig. 2). Mortality rate was decreased by rising of temperature. Materna *et al.* (1995) suggested that temperature influenced amphibian mortality. At 29°C temperature cannibalism was the highest and was the lowest at 35°C. Cannibalism might be another factor for larvae mortality. Das (1995) reported cannibalism might be responsible for the decrease of *Rana tigrina* larvae before metamorphosis.

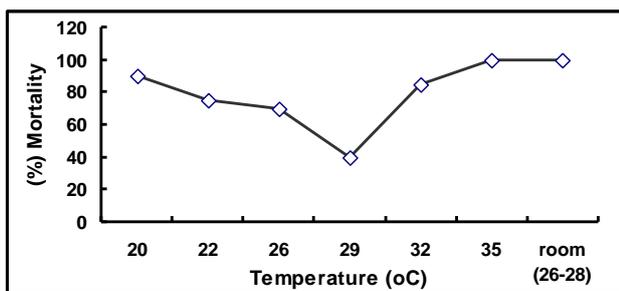


Fig.2. Trend of mortality observed at different temperature during study

Larval period in the laboratory: Larval period was influenced by temperature. It varied with the different temperatures. Larval period (up to absorbed tail) first completed in 29 days , 24 days , 18 days , and 25 days and maximum number of tadpoles completed their larval period in 52 days , 43 days , 42 days and 51 days at the temperature of 26°C, 29°C, 32°C and room temperature (26-28°C), respectively . It is interesting that no tadpole had showed completion of larval period at 35°C. This variation of larval period due to the variation of temperature has significant value in the life cycle of the frog. The first completion of larval period was the fastest (18 days) at 32°C and was the slowest (29 days) at 26°C. Similarly, tadpole at 32°C required the minimum number of days (42 days) for completion of the larval period of the whole lot of tadpoles whereas; tadpole at 26°C required the maximum number of days (52 days) for the larval period completion. Therefore, it is clear that high temperature required short time and low temperature required long time for completion of larval period. Martinez *et al.* (1994) showed that the larval of *Rana tigrina* at 22-25°C was slightly shorter than at 18-22°C. Barua (1982) found that the larvae of *Rana tigrina* took 42 to 52 days to complete metamorphosis at 27°C, to 32.7°C.

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