Journal of Indian Research (ISSN: 2321-4155)
Vol.1, No.4, October-December, 2013, 89-94.

Biological Science

MASS PRODUCTION OF FROG THROUGH INDUCED BREEDING AND GROWTH IN LABORATORY AND FIELD CONDITION – THE CASE OF INDIAN BULLFROG (*Rana tigerina*)

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ABSTRACT

Frogs are one of the most used and abused animal in field and laboratory setting. They are persecuted for varieties of reasons making many of their species vulnerable. As they are used in large number for laboratory work and for food, there is need for induced breeding and mass production at field level. With the objective of developing protocol for induced breeding and mass production, the study applied both experimental and field based methods such as inoculation of pituitary gland and induced breeding at laboratory to revealing limiting factors for frog farming at field level. The study revealed that frogs could be artificially bred in both the laboratory and field condition. Various factors which play greater role in such process are covered in this paper.

Keywords: Conservation, Frog, Growth and Development, Induced Breeding, Mass Production,spermiation,

INTRODUCTION

Nepal is richly endowed with diverse plants and animal species because of its varied topographical features ranging from 60 m to 8848 m altitude. This unique topographical features has created a conducive environment for amphibians particularly frog to be abundant in Nepal (IUCN, 1998). There are 53 species of frogs which are found from mountains to *terai* part of Nepal (*Shrestha*, 2001). The Indian bullfrogs (*Rana tigerina*) is the best known of the amphibians and quite common in Nepal and it has largely been used in research and scientific purposes. It is a widely used item in zoological practical classes in Nepal (*Timsina and Upadhaya*, 2001).

Many species of frogs are becoming endangered because of their over exploitation (*Shrestha, T. K.*, (1981), IUCN (1988), *Schleich H. and Kastle W.* (2002). It has been reported that the frog which are used in colleges and research centres in Nepal are manually collected from agricultural field or pond in India and Nepal. There is no scientific frog farming centre in India and Nepal (*Timsina and Upadhaya*, 2001).

Captive rearing of frogs has been proposed to protect populations from overharvest (*Warkentin, I. G.*, 2008). In nature, ovulation in the local species of frogs is triggered by rains at various times throughout the year except in the winter months. Mating and breeding of frogs in confined semi-natural conditions, as well as in concrete ponds, are quite successful. Controlled reproduction of the frog species has been successfully carried out by using GnRH analogue to induce spermiation, ovulation and mating. Spermiation was successfully induced by the intraperitonial injection of a single daily dose of GnRH over three consecutive days *(Pariyanonth and Daorerk*,1994*)*. Pariyanonth and Daorerk have

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also suggested that induced mating can be done by treating only the female frogs with the proper males in the breeding season. Fertilized eggs normally develop into tadpoles within 18-38 hours; the tadpoles later metamorphose into froglets within 28-36 days.

Frog farming has become quite popular and being practiced for long in many Asian countries such as Thailand. The demand for food to feed the rapidly increasing human population could help to raise farming to a commercial scale in the foreseeable future (*Pareta, P.* ,1978; *Schutt, S. L.* ,2009). Three species, *Rana tigerina, R. rugulosa* and *R. catesbeiana* are commonly farmed in many parts of Thailand. With the advances in biotechnology, frog farming can certainly be raised to the agro-industry level (*Pariyanonth and* Daorerk). *Campbell* (1999) emphasizes that frog husbandry includes three components: holding (temporary care of animals), rearing (for conservation and food purposes), and breeding (production of animals from captive-held stock).

The study on the induced breeding activities of the frog by experimental means and under laboratory and field condition is of great significance for scientific, ecological and food value. There has been a great demand for frog at various campuses of Tribhuvan University and other institutions for laboratory experiment of the science students in Nepal. Besides being a food value (eaten as Paha by villagers in the eastern mountain regions of Nepal), it has been reported that 3 to 5 star hotels are using frogs as delicious dishes to the customers (*Timsina and Upadhaya,* 2001). Excessive collection and disappearance of frogs may lead to the change in food chain of an agricultural ecosystem which in turn may cause outbreak of insect pest (*Shrestha,* 2001). Because of these impending conditions, a research work was conducted to establish experimental protocol on induced breeding activities of native species of frog (*Rana tigerina*) under laboratory condition which in turn can be applied to the field condition (CoE, 2012).

The objectives of the study were to assess the factors associated with frog breeding and growth in both laboratory and field condition.

MATERIALS AND METHODS

The study was based on both laboratory and field assessment which used both the primary and secondary data. Following methods and techniques were used in this research.

Site Selection and the field

A simple lab was set up near the pond located in Maheshpur VDC of Jhapa district in Nepal so that both the laboratory and outside study were carried out from the same place. The study on the induced breeding activities of the leopard frog (*Rana sphenocephala* or *Rana spp.)* was started from late July 2011. It took nearly one month to make arrangement of the scientific equipment including aquarium. The researcher carried out an observation tour of various colleges in Kathmandu and Jhapa districts to examine the use of frog in the dissection classes.

Collection of Frogs for Laboratory and Field study

The frogs collected in March and April were used for the experiment of the induced breeding. However, for the field level study (in the pond) the frogs were collected in June and July. For induced breeding, the study followed the method proposed by *Robert Rugh* (1968) in Experimental Embryology in which the removal of anterior pituitary gland from the roof of the mouth cavity is carried out. The pituitary gland was removed by exposing the brain and lifting it from the lateral side concentrating the eye on the posterior ventral part of the infundibulum where the pituitary gland is located.

In order to bring the frogs in the farming pond, the researcher carried out some manual practice. In this way, he collected about 50 adult male and female frogs and took them to the pond. In order to bring more to the pond, he used the cassette voice of the frogs recorded beforehand. This was done right at the mating time. He repeated this process for 2 days and had about 60 frogs gathered in this way.

Laboratory Study

The artificial fertilization of the frogs was carried out in the specially prepared laboratory in Jhapa district. Frogs were collected from a temporary pond located at Maheshpur VDC of Jhapa district. They were kept in an aquarium containing shallow water at laboratory temperature.

The female frogs of *Rana spp.* were collected from a pond and were found to be sexually matured as shown by the

presence of well developed ovary. The frogs were first anaesthetized so that the work could be carried out easily. By dissecting the female frog on their dorsal side locating from the lateral side of the brain, the pituitary glands were taken out.

Field Survey – Observation and Interview

Field survey was carried out regularly to know about different species and their status. Observation of the study areas ware carried out regularly by using several holistic and interdisciplinary ecological methods (*Michael, P.* 1989). The researcher observed laboratory classes of various colleges to examine the practice of dissection of frog by students. Another objective of the laboratory visit was to observe the amount of frogs used for the dissection purposes. The researcher also asked a number of local people and other people who were directly involved in frog farming or frog collection.

Data Interpretation and Synthesis

The information collected through laboratory and field assessment was analyzed and interpreted which helped in synthesizing the result of the work.

Materials

Various materials used for the experiment are rubber gloves, tray for dissection, air blower, syringe, collecting net, thermometer, petri dish, aquarium, dissecting box, collecting bottles, refrigerator, camera, glycerin, ether, distilled water etc.

RESULTS AND DISCUSSION

Laboratory Study

Inoculation of Pituitary Glands:

Along with 2cc. of medical distilled water four, five and one piece of pituitary glands were mixed separately and then inoculated to the lower abdominal quadrate of the sexually matured three different female frogs. In the first phase of the experiment, the inoculated frogs were then kept in a separate aquarium at 25°C and the time of ovulation was recorded. In the second phase of the experiment, the same number of pituitary glands were inoculated and the frogs were kept at 20°C and the time of ovulation was recorded. All the frogs were kept in common tap water with neutral pH and developing suitable environment. Air was supplied constantly by an electric air pump.

Five petri dishes with label N, 0.1N, 0.01N, 0.001N, and 0.0001N were prepared. Two pairs of testes from frogs with sperms were removed and changed to fine pieces in 10 cc.of tap water. The sperm mass was mixed properly manually putting in a test tube and was put into the petri dish "N" allowing it to settle down. By using a pipette, 1cc. of the sperm suspension from the N petri dish was taken out and mixed with 9cc. of tap water in petri dish "0.1N". In this way the concentration of the sperm suspension was decreased in other petri dishes.

Pituitary glands were extracted from the sexually matured frog (*Rana tigerina.)*. Five pituitary glands with 2cc of distilled water were inoculated to a big size female frog and was kept at 24°C in aquarium and left for 40 hours. In this time duration, it did not show any sign of ovulation which may be due to the inoculation of glands done inappropriately. While dissecting this frog, it was found that in the abdominal region there were some eggs. However, another one, which was inoculated with 3 glands, showed ovulation after 20 hours at 23°C. This may be the lowest number of the pituitary glands that has to be inoculated to result fertilization in an artificial way. However, it also depends on the variation of temperature range. In this experiment, the induced frog became sluggish and finally died after 6 days.

Table 1: Sources of pituitary glands

Date	Female Sps.	Pituitary Source (Male or Female)	No. of Pituitary	Temp. ^{0}C
June 2012	*Rana spp.*	Female	4/Female	24
July 2012	*Rana spp.*	Female	3/Female	25
August 2012	*Rana spp.*	Female	3/Female	23

Source: CoE (2012)

There is a dire need of ideal condition of time, chemical component in water and temperature for fertilization of normal eggs of the frog, *Rana spp.* Because of time constraint only one variable i.e. the amount of the spermatozoa was used. The temperature was kept as the temperature of the laboratory and eggs were to be stripped into the sperm suspensions within 30 minutes of the maceration of the testes.

Table 2: Result of the laboratory experiment

Date	Sps. Type	Pituitary Source (Male/Female)	No. of Pituitary Extracted	Size of Frog (in cm.)	Temp. Medium ^{0}C
June 29, 2012	*Rana spp.* (big)	Female	5	23	25
June 30, 2012	*Rana spp.*(small)	Female	3	15	24
June 30, 2012	*Rana spp.* (small)	Female	4	16	25-26
July 1, 2012	*Rana* spp.(big)	Big Male – 1 Big Female -1 Small – 2	4	27	22-23
July 1, 2012	*Rana spp.* (small)	From big Ovulating Female Frog	1	15	24-25

Source: CoE (2012)

The study has proved that when the frog is provided enough food before and after inoculation of pituitary glands, it can produce more eggs than the one which is not fed properly.

Table 3: Examination of number of eggs and insemination

Sperm Concentration	No. of Eggs	Inseminated in vitro (No. of eggs)	Inseminated in vitro (% of eggs)
N (Control)	50	44	88
0.1 N	50	46	91
0.01 N	50	10	20
0.001 N	50	36	72
0.0001 N	50	6	12

Source: CoE (2012)

The result of the artificial fertilization shows that the number of fertilized eggs is comparatively higher in N concentration of sperm than in others. By providing air regularly through the air pump and keeping the eggs at 25°C temperature, the researcher tried to examine the change in eggs.

Induced breeding conditions and parameters (i.e. temperature, time and number of pituitary glands) are different in different species of frog (*Rugh*, 1968). Frogs can reproduce at any time in the laboratory if it is provided appropriate temperature, water for habitat and food. Regarding its production, even the artificial fertilization and introduction of hormone is not necessary.

Farming at Field Condition

Based on the field data, the researcher developed a system for frog farming in an environment suitable to Nepalese context. The major components are dealt below.

Farming Ponds - Different types of ponds are required for different stages of frogs. For the tadpole stage, a separate but small pond or a tank of appropriate size should be managed. The pond should be properly fenced so as to confine frogs in the area (*Watabe, K.* 2010).

Bull frogs have a migratory nature and are susceptible to predation. A low, small mesh fence 3 to 4 feet high can be

erected around the pond serving to contain frogs and exclude such predators as cats, snakes and turtles. Some sunshine is desirable to promote algal growth, but too much may result in excessive algal growth which is detrimental to water quality. Low oxygen levels in the water will not encourage frogs and can make the pond rather smelly. For the local species, the stocking capacity of the pond is about 80-100 full grown frogs per m^2 (*Pariyanonth & Daorerk*).

Species Selection and Mating – In this study, Indian Bullfrog *Rana tigerina* was introduced as an appropriate species for scientific study. In the rainy season when breeding starts, the male frog comes to the breeding ponds. Hence they use their specialized calls to attract female mates. The male frog climbs on top of the female's back and clasp the female around her 'waist' in what is called amplexus, and the eggs are fertilized in the water as she lays them in masses.

Eggs and Tadpoles- The frogs lay many eggs, but because of the high mortality rate, only a small percentage of eggs can reach to the tadpole stage. The egg masses were immediately transferred to the small pond, using a scoop to handle them. Then, the fertilized egg began to divide (*Vidyarthi,* 1998).

After few days, each cluster in the pond became a living mass of tadpoles. Tadpoles are scavengers, eating anything from table scraps to water moss. Shortly after hatching, the tadpoles fed on the remaining yolk. After about six to nine weeks, the tadpoles started to absorb their tails and grow legs and arms. Tadpoles cannot thrive on polluted or toxic water. Tadpoles like spinach, but needs to be boiled and drained properly. When water gets dirty, feeding frogs should be slowed down and water should be replaced (*Rugh,* 1968).

Frog – In the field, the raised frogs ate snails, bugs, beetles, mosquitoes etc. Since, the frog demands live food, the management of food for the growing frogs was the most difficult aspect in frog farming. As frogs and their tadpoles are cold-blooded animals, they have a slow growth rate. Their actual growth rate is directly related to the length of the growing season and the amount of food that was available (MDC). However, the study was carried out till froglet stage since transformation to frog takes several months to year (*CoE,* 2012).

Harvesting - The frog should be harvested before they go for aestivation or hibernation. Common techniques for harvesting are pole and line angling, spearing or gigging, or grabbing by hand. All are labour intensive.

Many frog farms can be established at natural marshy areas, swamps or shallow ponds which have abundant food and habitat suitable to the needs of wild frogs. The frogs usually are left to raise themselves. During the field visits, the researcher observed many of such sites in Jhapa district. Frog farming depends on a number of factors such as costs of land and feed, fencing and lighting costs, occurrence of disease, cannibalism, climate, predation etc.

SUMMARY AND CONCLUSION

There is a lack of scientific information regarding frog farming in Nepal. Therefore, referring to the academic and practical texts, the researcher himself designed some of the techniques that could be employed for frog farming. Frog hunters capture frogs in wild and sell them to colleges and hotels when they see any demand. However, frog farming seems to be feasible and viable economic option in many parts of the country.

There is possibility and potentiality of mass production of frog in the laboratory and field condition in Nepal. However, despite the hypothesis taken before hand, time has still not been ripe enough to kick off frog farming at campus level. This is not because of the lack of possibility, but more because of lack of any system for continuity and regular supervision. The work is also geared to improve our understanding of the intricate relationship among human beings, environmental degradation and vulnerability in frog species so that it could contribute to the improvement of practices of frog farming in Nepal. The study has also partially contributed in carrying out induced ovulation for breeding activity so that commercial production of the frogs could be carried out. This research has opened up the door for the further study of commercial frog farming in our context.

ACKNOWLEDgEMENT

I am very much grateful to the Director and staff of Centre of Excellence for PhD Studies (CoE) who provided me this opportunity to pursue scientific research work on 'Developing the System of Mass Production of Frog in Laboratory and Field Condition in Nepal' by providing small grant and moral support during the study. I would like to express my sincere gratitude to Dr. K. R. Khambu, Dr. Ram B. Khadka and Dr. Herman Schleich for giving feedback and suggestions to my work.

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