Studies on anuran development : An Experimental Analysis Of Larval Growth and Metamorphosis of *Rana limnocharis* Wiegmann, in Relation to Certain Environmental Factors

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CERTIFICATE

I, the undersigned, certify that this thesis entitled "STUDIES ON ANURAN DEVELOPMENT : AN EXPERIMENTAL ANALYSIS OF LARVAL GROWTH AND METAMORPHOSIS OF *Rana Limnocharis* Weigmann, IN RELATION TO CERTAIN ENVIRONMENTAL FACTORS", submitted by Mr. ASHISH GUPTA for the degree of Doctor of Philosophy of the North-Eastern Hill University has been compiled under my supervision during the period 1984-87. He has been duly registered for the award of Ph.D. degree and the thesis presented is worthy of being considered for the award of the Ph.D. degree. This work has not been submitted for any degree to any other university.

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INTRODUCTION

The utility of frogs and toads is known since times immemorial. They have been used from the dawn of the scientific era for a number of discoveries ranging from the disccvery of Galvanic currents by Galvani in 1780 (quoted in Ronan, 1983) to pregnancy diagnosis tests in this century (Cochran, 1961). They are used world over for imparting fundamental zoological knowledge as well as for research in the field of vertebrate anatomy, physiology, genetics and development (see Cochran, 1961; Nace, 1968; Heusser, 1974 among others). Among vertebrates they are first animals used for investigation on the phenomenon of differentiation, and also they are the first vertebrates in which cloning was first investigated and its use was demonstrated. Economically, the use of frogs as food and for medicinal purposes, such as, for heart ailments, wounds and for food poisoning has been known in certain countries for example China and South East Asia from Prehistoric days (Henderson, 1864). In recent years they are also used as gourmet food items in many western countries.

The frogs and toads belong to Order Anura, which is the largest living Order of Class Amphibia. They are widely distributed all over the world except snow clad mountains and waterless deserts. They inhabit fresh water systems and their surroundings. One species *Bufo marinus* has been reported from marine waters in South America (Bradoo, 1986). Some species are completely aquatic

such as *Rana cyanophlyctis* and *Xenopus laevis* and some prefer to live mostly on land such as *Bufo melanostictus*, but great majority of anurans are amphibious in habit; but all of them breed in water.

In North-Eastern India, which is endowed with tropical and subtropical ecosystems, has varieties of streams, rivers, lakes, hills, mountains and humid climatic conditions ranging from hot plains to cold weather at higher altitudes. The region is gifted with varied types of forest systems and a huge assemblage of rich and diverse flora as well as fauna. According to recent reports more than 124 species of Anura grouped in 25 genera and 8 families have been described from the Indian subcontinent (see Pillai and Murthy, 1986; Kiyasetuo, 1986 unpublished, among others). Pillai and Chanda (1976) compiled information that as many as 40 anuran species have been reported from North-Eastern region alone. And out of these, the state of Meghalaya itself has 30 anuran species, belonging to 6 families. These numbers are ever increasing as more and more reports are flowing in.

The frogs and toads have always been used as models for various types of morphological, physiological, developmental as well as ecological investigations. Though we have in this country so many types of anuran species, we do not have information on biology, development, genetics and ecology of even most common species. In the first world conference on the export of frozen

frog legs vis-a-vis environmental considerations organised by Marine Products Export Development Authority (MPEDA) and Central Inland Fisheries Research Institute (CIFRI), Barrackpore at Calcutta in 1986, deep concern was expressed on the indiscriminate exploitation of anuran fauna of this country. The frogs and toads are used for educational purposes in schools, colleges and a number of research institutions all over the country. Millions of tons of frozen frog legs are exported from this country through MPEDA to Western countries (Table 1.1). It is estimated that in 1980 alone about 18 million tons of frogs were used for educational purpose where as about 60 million tons of frogs were used for export purpose. A comparison of the trends on their utilization since 1956 (Fig. 1.1) reflects that educational sector utilizes 1/3 of that exported by MPEDA. As a result of it their population is reaching a low ebb raising an alarming concern. It was felt though the practices to exploit anuran fauna cannot be completely stopped, it is possible to raise more anurans by culture. Before undertaking such programme, it is necessary to have basic informations on various aspects of biology, physiology as well as ecology of these animals.

The frogs and toads from an important component of our ecosystem. They play a vital role as intermediate trophic members as well as predators in the following food chain.

Plants>Invertebrates>Frogs/toads>Birds and Reptiles>Mammals

Besides being an important unit in ecological cycles and food chains it has been estimated that they protect our crops from innumerable pests and predators. Increasing trends of weights of undestroyed pests in relation to weights of frogs killed since 1963 have been shown in Table 1.2. Their indiscriminate capture badly affects the agro-ecosystem as the crops are damaged by many pests and predators, which are otherwise removed in a natural way by frogs and toads.

Though the adult frogs have their own role, their larval stages are nevertheless very important in the ecosystem. Their presence in large numbers in the fresh water ecosystems during monsoon periods provides an excellent opportunity to examine the impact of transient consumers on the dynamics of unmanipulated aquatic ecosystems (Seale, 1980). They form an an important link in the food chains of natural fresh water ecosystems feeding on algae and plankton and being preyed upon by naids and larvae of other aquatic insects, fishes etc. (Heyer, 1973; Heyer and Muedeking, 1976). They also have a great biological significance as the larvae exploit food sources which otherwise could have been completely inaccessible. Ecologically they are referred to as energy gathering phase. The abundance of the adult population depends on the chance of successful metamorphosis before they are killed by unfavourable conditions or ravages of predation and disease.

In recent years increasing interest is being shown on investigations on anuran tadpoles such as their food and feeding habits (Sabnis and Kuthe, 1978), changes in food niche during development (Christian, 1982) suspension feeding mechanisms (Seale, Hoff and Wassersug, 1982; Viertel, 1984), morphology of filter apparatus (Viertel, 1985), interspecific and intraspecific competiton (De Benedicts, 1974; Steinwascher, 1978), density dependent aspects of metamorphosis (Wilbur, 1976, 77; Dash and Hota, 1980),interactions of food levels and larval density (Hota and Dash, 1981), thermal adaptations (Brattstrom, 1970; Claussen, 1973), daily and seasonal variations in thermal tolerance (Willhite and Cupp, 1982; Floyd, 1983) and daily cycles of aggregative behaviour (Beiswenger, 1977).

While the taxonomy of the tadpoles has contributed to a better understanding of amphibian systematics (Starrett, 1973; Khan, 1982), the ecological and ecophysiological studies on the tadpoles have helped us in understanding their bioenergetics, adaptation and distribution, their role in the ecosystem and maintainance of larval stages of the tadpoles. All these informations are needed for investigations on the growth and metamorphosis as well as culture possibilities of anuran species.

Rana limnocharis Weigmann, the streaked frog is a very common species found in Shillong and surrounding hills of Meghalaya. It is a widely distributed species of the Indian

subcontinent as well as in Eastern Tropics, both on plains and hills (Satyamurti, 1967). An earlier study on its embryonic limiting temperatures (Roy and Khare, 1979) indicated that this species has a remarkably wide range of thermal tolerance levels. It was felt that an ecophysiological study on different aspects of the tadpoles of this species may also throw light on its adaptational and wide range distributional pattern, in addition to generating information which may be helpful in its maintenance and culture. Needless to say that besides being a common edible species among trible communities, *Rana limnocharis* is one of the most suitable species for educational and research purposes at the North-Eastern hills of India.

For the present thesis the developmental pattern of *Rana limnocharis* has been carefully observed and following three aspects viz : (1) Effect of food, (2) Effect of density, (3) Effect of temperature, on the growth and metamorphosis of tadpoles have been thoroughly investigated.

The knowledge of developmental pattern of any species is a very fundamental need for experimental investigations. As certain stages described by Roy and Khare (1978) needed some refinement, the work was started with such a description. The changes in the body weight with time have been considered by certain workers, an important parameter for studies on the growth of the tadpoles (Dash and Hota, 1980; Mishra and Dash, 1984; Petranka, 1984). Also

certain morphometric measurements such as total length, body length and tail length of the tadpoles are important features to be noted during their growth and metamorphosis.

With the study of feeding habits of the tadpoles it is possible to know their food preferences (Sabnis and Kuthe, 1978; Christian, 1982). It is also possible to analyse the distribution of these items in the system which they inhabit (Farlowe, 1928; Sahu, 1981). For culture, growth and maintainance, the need for artificial food items has been felt for a long time. Thus in addition to analysis of gut contents of successive larval stages, an experimental analysis of the effect of different artificial food items on growth and development of larvae has been carried out.

The density of the larvae in any circumscribed space has profound effect on their growth and metamorphosis (Wilbur, 1976,77; Dash and Hota, 1980; Semlitsch and Caldwell, 1982; Sokol, 1984); but the effect may vary from species to species. For example in a single population of salamander *Ambystoma maculatum* may metamorphose between weights of 0.24 g and 2.34 g over a period from 57 to 144 days after hatching (Wilbur and Collins,1973). Also there are several views explaining the negative effects of density on growth of tadpoles. For example, the negative effects may be due to competition for food (Brockelman, 1969; De Benedictis, 1974; Dash and Hota 1980), growth inhibitors released by large growing

larvae(Licht, 1967) and behavioural interactions (Gromko et al. 1973; John and Fenster, 1975). As such this issue has been examined intensively in this investigation.

Of all the abiotic factors temperature has a very important role to play in the ecophysiological and internal regulatory mechanisms of the organisms and this in turn seems to have played a vital role in their adaptation, distribution and evolution (Zweifel, 1968; Bachman, 1969; McLaren and Cooley, 1972; Brown 1975; Townsend and Stewart, 1986). The thermal tolerance and adaptation of the tadpoles seem to have an enormous effect on development, growth and distribution.

How an animal acclimates itself to varied ranges of temperatures is an intriguing phenomenon. Temperature is one important environmental factor effecting the survival and efficiency of organisms within the environment. Limiting effects of temperature may be modified by short term physiological adjustment or acclimation (Fry, 1958; Prosser, 1958). Thermal acclimation is measured in terms of changes in thermal tolerance levels (critical thermal maxima and critical thermal minima) and is defined as "the arithmetic mean of the collective thermal points at which locomotary activity becomes disorganised and the animal loses its ability to escape from conditions that will promptly lead to its death". Many workers such as Brown (1969), Cupp (1974), Dunson (1977) and Sherman (1980) have analysed that

high thermal tolerances of anurans in general reflects their ability to life in temporary ponds through permitting maximization of body temperature and hence developmental rate. The ability to adjust their critical thermal levels and associated physiological adjustments at different temperatures is also an added advantage to anurans as it enables them to become metabolically more efficient at new temperatures which might if behavioural responses failed be lethal (Brattstrom, 1962). In anurans the CTMax or CTMin have been found to change at different embryonic stages (Zwiefel, 1977; Kuramoto, 1978)as well as at different larval stages (Cupp, 1974; Sherman, 1980; Dupre and Petranka, 1985). Not only at the critical stages, but also at different times of the day these tolerance levels differ in the larvae (Willhite and Cupp, 1982) as well as adults (Mahoney and Hutchison, 1969; Johnson, 1972a,b).

Thus a thorough investigation has been carried out on the thermal tolerance levels and their variations at different larval stages as well as at different timings of the day. The influence of thermal acclimation on these levels has also been examined. How these factors effect the development, growth and metamorphosis of the larvae of *Rana limnocharis* has been investigated.

It is hoped that the results of the present investigation will not only give a better insight on food, density and temperature relations of the larvae of *Rana limnocharis* in their ecophysiological and developmental cycles, the broad conclusions

may be of help in formulating the culture and maintenance of other species of anurans as well.