

# **DEVELOPMENT OF AUDITORY SYSTEM AND ACOUSTICALLY MEDIATED BEHAVIOUR IN ANURANS**

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# **SYNOPSIS**

## INTRODUCTION

The progress from aquatic to terrestrial living was one of the most important events in the evolution of higher vertebrates. In the ontogeny of amphibians, the change from fish to tetrapod is faithfully repeated generation after generation. An aquatic larval period precedes the terrestrial period. Frogs and toads undergo extensive change and after metamorphosis approach the amniote type. Their locomotor and respiratory organs become entirely remodeled. After metamorphosis they develop new nutritional habits and accordingly the digestive system suffers radical change. In fact, the entire body becomes rebuilt, with the exception of the sex glands, which are little affected and attain maturity only after metamorphosis.

In view of the general, physiological and evolutionary importance of metamorphosis in the amphibians, it is surprising that it has never been investigated in a systematic way. One of the important organ which has not received due attention is the **EAR** or the **AUDITORY SYSTEM** during metamorphosis, though the morphological and functional change from underwater hearing in premetamorphic aquatic tadpoles to aerial hearing in adult terrestrial frogs accompanied by replacement of the larval accessory apparatus by entirely new tympanic organ of the adult are spectacular. Thus, the development of the amphibian ear and its sound perception system represent an astounding example of adaptation to changing environmental conditions.

It is with this view, the present work has been formulated for proper understanding of the morphological and physiological development of the auditory system as well as the development of acoustically mediated behaviour in *Limnonectes limnocharis*.

## MORPHOLOGICAL AND FUNCTIONAL BACKGROUND

When vertebrates first ventured onto land, they had to adapt to terrestrial condition in various ways. This holds true also for hearing, because the properties of sound are quite different in air than in water. Fishes possess inner ear with otolith organs. Since water has approximately the same density as fish, sound waves in water run through the fish and vibrate its body. A fish with its inner ear thus vibrated. Airborne sound of normal intensity, however, will not bring the animal's head into vibration. Therefore, the air filled middle ear has been developed, the components of which such as the tympanum and the columella bridge the impedance mismatch between the airborne sound and the fluid filled inner ear.

These changes, that had occurred during vertebrate transition to land, are repeated today in the development from the tadpole to frog. Tadpoles possess an inner ear but no middle ear. At later stage, lungs develop. The lung becomes connected to the inner ear through the round window. It has been argued, that this connection supports tadpole hearing similar to the way swimbladder support hearing in fish. The middle ear begins to develop at early metamorphosis, completion of development may take 2-12 months or more depending upon the geographic location and climate of the breeding area. The connection between the lung and inner ear ends at metamorphosis and the presumed input site for sound in the tadpole- the round window now becomes the release site of the inner ear for the sound which comes in via the columella through the oval window. Even when the middle ear has developed fully in the young froglet, the size of the head is considerably smaller than in the adult frog. Since the frog ear acts as a pressure gradient receiver which reacts according to the pressure difference between the sound impinging from the outer side of the tympanum and

the sound impinging on the tympanum's inner side via the eustachian tube, thus the size of the head should also have a considerable impact on the hearing ability of the frog.

## CURRENT STATUS

Most of the reports on amphibian auditory system and anuran acoustic communication has been from adult full grown frogs ( Capranica 1976; Gerhardt 1982; Hetherington and Lombard 1982; Littlejohn 1977; Roy and Elepfandt 1993; Ryan 1986; Wells 1977; Wever 1973 ). The reason may be the following :

1. The biophysics of hearing in frogs is so different from that of mammals and also very complicated, that investigations have been concentrated for a long time on understanding the adult amphibian hearing.

2. The difference of underwater sound from the airborne sound necessitates a different experimental set up than for studies on airborne sound.

The few literature available for work on premetamorphic or early postmetamorphic stages are mostly on either morphological features of the ear of the transforming larva ( Paterson 1949; Roy and Elepfandt 1990; Sedra and Michael 1959; Spaeti 1978; Weisz 1975; Witschi 1949 ) and that too, all these works are mostly on **Xenopus** or on kin recognition in tadpoles ( Blaustein and O' Hara 1982; Blaustein, Bekoff and Daniels 1987; Blaustein and O' Hara 1988; Waldman and Adler 1979; Waldman 1981 ).

## AIMS OF THE PROPOSED PLAN OF WORK

The aim of the proposed plan of work is to investigate the ontogeny of the auditory system, development of hearing and the behavioural responses exhibited to define sound stimuli. Amphibians provide interesting model for changes that may have occurred during

vertebrate evolution from water to land. So far however, amphibian hearing has been examined in adults only. Morphologically tadpoles possess an inner ear but the middle ear that is found in adult anurans develop not before metamorphosis. Hypotheses have been made about how tadpoles might hear, but experimental evidence that they do hear is lacking. Also, according to the present model of hearing in postmetamorphic anurans, hearing abilities should change with the size of the head. The project is aimed at testing hearing abilities of tadpoles and young froglets and by this, assessing the present model of hearing in *Limnonectes limnocharis*.

Thus the basic questions to be answered are :

1. At what stage during metamorphosis do the tadpoles or the young froglets begin to hear ?
2. What behavioural responses do these animals exhibit to define sound stimuli ?
3. What are the related morphological changes in the sound conducting system ?

## **EXPERIMENTAL PROGRAMME**

It will be essential to mention at this juncture why *Limnonectes limnocharis* has been chosen as the particular species to be studied :

1. In order to study the morphological details of the auditory structure, the normal table of development for this species will be needed. The normal table of development has already been studied ( Roy and Khare 1978 ).
2. *Limnonectes limnocharis* colony has to be set up. For this the frogs have to be induced bred, fertilized eggs have to be reared at their particular preferred temperature and finally the animals have to be fed with the food of their preference. Data on all these aspects are already available ( Khare, Kumar and Roy 1981; Roy and Khare 1979; Roy 1987; Roy 1990 ).

3. For experiments on acoustically mediated behaviour, a prior knowledge of the adult call pattern will be essential. This is already recorded and analysed ( Roy and Elepfandt 1993 ).

## **A. RAISING AND MAINTAINING BREEDING COLONY UNDER NATURAL AND LABORATORY CONDITION**

For any in depth study of neuroethology, it is of utmost importance to have a good animal facility. This would give a ready supply of animals needed for experimentation of any age, sex and weight. Continuous observation of the animals kept in the facility will also lead to a better " feeling " for the animals and their behaviour.

### **i. Colonies under natural conditions**

A water reservoir of 3 m x 3 m will be dug with 2 ft. depth having a slope. This will be surrounded by 2 m wide grass path. The entire area will be covered by wire netting 8 ft. high. Two 100 w lamps will be lit at night which will trap insects as food for the animals.

Initially the experimental species will be collected immediately after their hibernation period. Some animals will be left undisturbed to breed naturally, while others will be induced bred to obtain fertilized embryos.

### **ii. Colonies under labortaory conditions**

Clean big aquariums with proper lighting, heating ( if needed ) and supply of aerated recycled water has to be set up. Initially a large variety of food will be tried, as the animals may show preference for a particular food item. This may vary according to the prey as well as the predator size. Once the preferred food item is known these food have to be reared under natural conditions. Extra care and experience will be needed to handle sick and weak animals for long period during behavioural testing in isolated conditions.

Time frame - 6 months.

## **B. MORPHOLOGICAL STUDY OF THE TRANSFORMING LARVAL AUDITORY SYSTEM DURING METAMORPHOSIS**

The different larval stages after measuring their head trunk length, tail length and head width will be fixed either in mercuric chloride, trichloroacetic acid and formalin or in Zenker fixative. The former fixative will be used for tadpoles upto metamorphosis stage and the later for young metamorphosed and adult frogs. Immobilised, perfused animals will be fixed for 48 hours. After fixations the animals will be washed in 4% lithium sulphate and water for 24 hours respectively. Decalcification will be done in EDTA of pH 7.4 at 37° C. After decalcification the specimens will be washed once again in 4% lithium sulphate and water respectively.

Cryostat sections of the head region both in saggital and frontal plane will be prepared. Sections will be stained by trichrome stain for differential staining of the cartilaginous and calcified structures of the auditory system. This study will give the total histomorphological picture of the developing sound conducting apparatus.

Time frame - 7 to 12 months.

## **C. STUDY OF THE DEVELOPMENT OF ACOUSTICALLY MEDIATED BEHAVIOUR IN METAMORPHOSING TADPOLES**

In view of the general physiology and evolutionary importance of metamorphosing frogs, it is surprising that this has never been investigated in a systematic manner. Hearing has been investigated in adult full grown frogs. Behavioural responses of the newly

metamorphosed anurans is still poorly understood. Conclusive experiments are needed in order to elucidate the tadpole's ability to hear. Comparative morphological studies combined with the analysis of function will provide the data necessary for understanding the evolutionary significance of morphological features. The emphasis will be on the following questions :

- a. At what stage during metamorphosis do tadpoles or young froglets begin to hear ?
- b. What behavioural responses do these animals exhibit to define sound stimuli ?
- c. What are the related morphological changes in the sound conducting apparatus ?

For answering these questions, extensive phonotaxis experiments will be conducted in acoustically isolated chambers.

#### **D. WHETHER SPECIES SPECIFIC CALLS ARE LEARNED OR INNATE ?**

This part of the work can only be started after the breeding colonies are successfully set up. Fertilized eggs will be obtained by induced breeding. 50 % of the fertilized eggs will be raised in acoustically isolated environment. The rest 50 % of the eggs will be raised along with the adult colony. The following will be observed and recorded :

- a. When do the young metamorphosed froglets give the first call ?
- b. Do the call parameters change as the animals grow old ?
- c. What different types of calls do they emit - related to their behaviour pattern ?
- d. Is there any differences between the calls emitted by the 2 groups - one raised in isolation and the other in colony ?

**Time frame - 25 to 36 months.**

## REFERENCES

- Blaustein, A. R. and O' Hara, R. K. 1982. Kin recognition in *Rana cascadae* tadpoles : Maternal and paternal effects. *Anim. Behav.* 30 : 1151 - 1157.
- Blaustein, A. R. , Bekoff, M. and Daniels, T. J. 1987. Kin recognition in vertebrates ( Excluding primates ) : Empirical Evidence : in *Kin recognition in Animals* ( Edt. ) Fletcher, D. J. C. and Michener, C. D. pp 287 - 357.
- Blaustein, A. R. and O' Hara, R. K. 1988. *Hyla regilla* and *Rana pretiosa* tadpoles fail to display kin recognition behaviour. *Anim. Behav.* 36 : 946 - 948.
- Capranica, R. R. 1976. Morphology and physiology of the auditory system in Frog Neurobiology ( Llinas, R. and Precht, W. eds ). pp 551 - 575.
- Gerhardt, H. C. 1982. Sound pattern recognition in some North American tree frogs ( Anura : Hylidae ) for mate choice. *Ann. Zool.* 22 : 581 - 595.
- Hetherington, T. E. and Lombard, R. E. 1982. Biophysics of underwater hearing in anuran amphibians. *Journal of Experimental Biology.* 98 : 49 - 66.
- Khare, M. K. , Roy, D. and Kumar, A. 1981. Induced breeding in two Indian frog species. *Proc. Natl. Acad. Sci. India.* 151 (B) II : 187 - 189.
- Littlejohn, M. J. 1977. Long - range acoustic communication in anurans : an integrated and evolutionary approach in *The Reproductive Biology of Amphibians* ( eds ) Taylor, D. H. and Guttman, S. I. ( New York : Plenum Press ) pp 263 - 294.
- Patterson, N. F. 1949. The development of the inner ear of *Xenopus laevis*. *Proc. Zool. Soc.* ( London ) 119 : 269 - 291.
- Roy, D. and Khare , M. K. 1978 Normal table of development of *Rana limnocharis*

- Wiegmann. Proc. Natl. Acad. Sci. India. XLVIII : 5 - 16.
- Roy, D. and Khare, M. K. 1979. The influence of embryonic limiting temperature on the development of *Rana limnocharis* Wiegmann. Biol. J. Linn. Soc. London. 11 : 279-287.
- Roy, D. 1987. Food and feeding habits of *Rana limnocharis* Weigmann, the common frog species of Meghalaya, India. Asian J. Expl. Sci. 3 : 1 - 9.
- Roy, D. 1990. Annual breeding cycle of *Rana limnocharis* in the northeastern hills of India. Proc. Nat. Acad. Sci. India. 60 (B) III : 363 - 365.
- Roy, D. and Elepfandt, A. 1990. Analysis of the middle ear development in the clawed frog, *Xenopus laevis*. Verh. dt. Zool. Ges. 83 : 469 - 470.
- Roy, D. and Elepfandt, A. 1993. Bioacoustic analysis of frog calls from northeast India. J. Bioscience. 3 : 381 - 393.
- Ryan, M. J. 1986. Factors influencing the evolution of acoustic communication : biological constraints. Brain Behav. Evol. 28 : 70 - 82.
- Sadra, S. N. and Michael, M. I. 1959. The ontogenesis of the sound conducting apparatus in salientia. J. Morphol. 104 : 359 - 375.
- Spaeti, U. 1978. Development of the sensory systems in larval and metamorphosing European grassfrog (*Rana temporaria* L. ). Journal of Hirnforschung. 19 : 543 - 575.
- Waldman, B. 1981. Sibling recognition in toad tadpoles : the role of experience. Z. Tierpsychol. 56 : 341 - 358.
- Waldman, B. and Adler, K. 1979. Toad tadpoles associate preferentially with siblings. Nature. 282 : 611 - 613.

- Weisz, P. B. 1975. The development and morphology of the larva of the South African clawed toad *Xenopus laevis*. J. Morphol. 77 : 163 - 217.
- Wells, K. D. 1977. The social Behaviour of anuran amphibians. Anim. Behav. 25 : 666 - 693.
- Wever, E. G. 1973. The ear and hearing in frog, *Rana pipiens*. J. Morphol. 141 : 461 - 478.
- Witschi, E. 1949. The larval ear of the frog and its transformation during metamorphosis. Zeitschrift fur Naturforschung. 4b. 230 - 242.