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 discovery 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 form 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 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 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.

TABLE 1.1

ANNUAL EXPORTS OF FROGS LEGS AFTER 1963

(ABDULALI, 1985)

YEAR	QUANTITY OF FROZEN FROGS LEGS EXPORTED TONNES	MINIMUM* QUANTITY OF FROGS KILLED IN TONNES	FOREIGN EXCHANGE EARNED Rs.	EXPORT RATE Rs./Kg. f.o.b.
1963	514.00	1542.00	3192000	6.21
1964	332.00	986.00	1650000	4.96
1965	443.00	1329.00	2604000	5.88
1966	557.00	1671.00	5576000	10.01
1967	786.00	2358.00	8817000	11.21
1968	425.31	1275.93	4891310	11.50
1969	854.37	2563.11	11889563	13.91
1970	2544.87	7634.61	32899364	12.92
1971	1451.14	4353.42	13774273	9.49
1972	1823.48	5470.45	21709398	11.90
1973	2697.60	8092.80	44878893	10.63
1974	1453.96	4361.90	28651727	19.70
1975	1317.48	3952.45	27982525	20.70
1976	3169.88	9509.65	77969621	24.59
1977	2834.20	8502.61	65966878	23.27
1978	3570.00	10710.00	84300000	23.36
1979	3764.00	11292.00	87200000	23.15
1980	3095.00	9285.00	73200000	23.06
1981	4368.00	13104.00	11960000	25.37
1982	2271.00	6813.00	55453406	24.40
1983	3658.00	10914.00	-	-
1984	2500.00	-	-	-
1985	2500.00	-	-	-

*excluding spoilts & rejects.

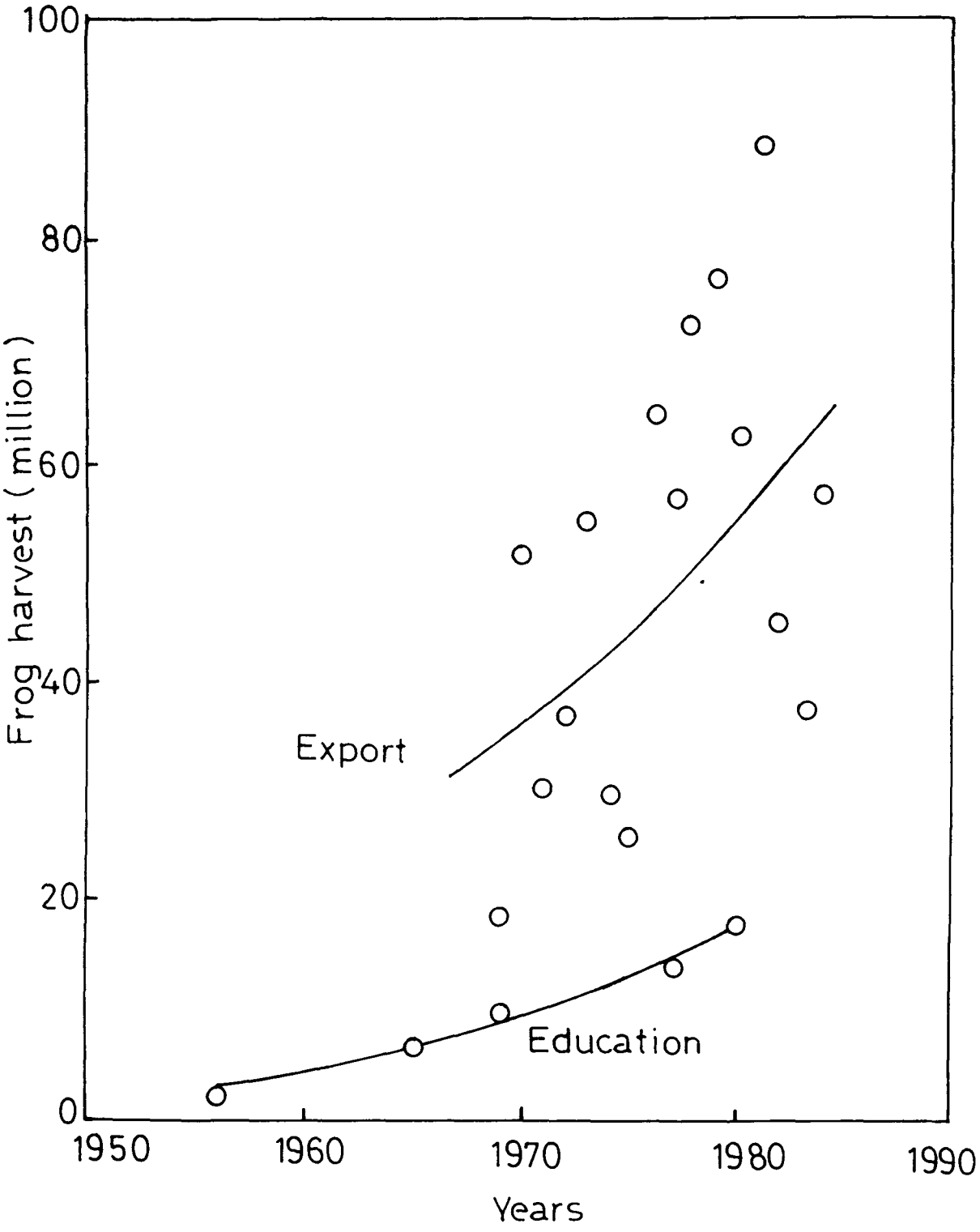


FIG. 1.1 INCREASING HARVEST OF FROGS BY EDUCATION AND EXPORT SECTORS DURING THE LAST 30 YEARS. THE SYMBOL O REPRESENTS THE LEVEL OF FROG EXPORT INITIATED BY ALLEN T SHERMAN (USA) (AFTER ABDULALI, 1985).

TABLE - 1.2

ESTIMATED WEIGHTS OF UNDESTROYED PESTS (IN TONNES) IN DIFFERENT YEARS 1963-78 (ABDULALI, 1985)

YEAR	WT. OF FROGS KILLED IN TONNES AS PER TABLE 1.1	TOTAL AMOUNT OF FROG FOOD REMAINING UNDESTROYED.	CRABS	INSPECTS	INSECT LARVAE	OTHER ITEMS
1963	1542.00	18141.12	11755.44	1981.01	2576.04	2757.45
1964	996.00	117176.40	73930.30	12795.66	16639.05	17810.81
1965	1329.00	15635.28	1014.13	1707.37	2220.21	2376.56
1966	1671.00	19658.76	12738.87	2146.74	2791.54	2988.13
1967	2358.00	27741.12	17976.24	1639.19	2938.24	4216.65
1968	1275.93	15010.92	9727.10	3292.85	2131.55	2281.66
1969	2563.11	30154.32	1953.92	9808.22	12754.29	14583.45
1970	7634.61	89818.92	5820.66	5592.86	727.77	13652.47
1971	4353.42	51216.72	33188.43	7027.92	9138.87	7784.94
1972	5470.45	64358.28	41704.16	10396.87	13519.74	9782.45
1973	8092.80	95209.44	61695.71	5603.75	7826.93	14471.83
1974	4361.90	51316.44	33253.05	5077.74	6609.93	7800.10
1975	3952.45	46499.52	30131.64	12217.74	15886.71	7067.91
1976	9509.65	111878.28	72497.12	10923.36	14204.37	17005.50
1977	8502.61	100030.80	64819.96	13759.20	17892.00	15204.68
1978	10710.00	126000.00	81648.00	-	-	19152.00

REVIEW OF LITERATURE

1- FOOD AND FEDDING HABITS

When we look back into the works done earlier it is revealed that the study on various aspects of food such as analysis of gut contents, filter feeding mechanisms, morphology of filter apparatus, energy estimations and artificial diets of anuran tapoles started as early as in late 19th century. Work done on some of the aspects studied in the present investigation has been described below.

GUT CONTENTS AS INDICATORS OF HABITAT

One of the earliest reports on the food and feeding habits comes from the worksof Dickerson (1906). He reported that the tadpoles of the green frog , *Rana clamitans* were herbi - vorous. Smith (1916) observed that tadpoles of *Oeidozya laevis* and *O. lima* fed on mosquito larvae and small tadpoles. In the year 1928, Farlowe reported that the tadpoles of *Rana clamitans* fed on algae. According to her the analysis of the gut contents provides a clear picture of the algal forms present in the particular habitat. Noble (1931) reported that tadpoles in general show greater food preferences than the adult frogs and toads, for some are exclusively vegeterian, others carnivorous, while majority take a mix diet. However Hora in the year 1934 reported that tadpoles of *Rana afghanus* fed on slimy matter found over the rocks and stones under the swift water currents.

Bragg (1940) described the tadpoles of *Bufo congnatus* as being partly scavangers while Pope (1947) analysed the gut contents of the tadpoles of *R. clamitans* and found them to contain diatoms, algae and minute quantities of small animal forms.

In early sixties, Kamat (1962) analysed the gut contents of some tadpoles (species not mentioned) from certain small ponds in India, while Dickman in the year 1968 reported that tadpoles can ingest epiphytic algae. Calef (1973) however reported that tadpoles can ingest epibenthic algae. Altig and Kelly (1974) investigated the indices of feeding in 13 anuran species, based on the gut characteristics. They found that carnivorous tadpoles had shorter and less voluminous guts than those of herbivorous tadpoles and different species have different feeding abilities.

Sabnis and Kolhatkar (1977) studied the food preferences of *Rana cyanophlyctis* tadpoles in India and found them to mainly feed on spirogyra species and other zooplanktonic forms. In the same year Wilbur described tadpoles to be omnivorous, while Das in the year 1979 reported that tadpoles of *Rana hexadactylus* fed on aquatic vegetation. Mallick et.al. (1979,80) and Mallick and Mallick (1981) studied the food and feeding habits of some salient tadpoles. They reported that besides vegetarian food habits tadpoles of *Rana maculatus* exhibit carnivorous and cannibalistic habits.

Dash and Hota (1980) observed tadpoles to be omnivorous, and in the same year Seale and Beckvar found that tadpoles have the ability to ingest blue green algae. Mohanty - Hejmadi and Dutta (1981) reported that tadpoles of *Rana tigerina* predate over *Bufo melanostictus* larvae repeatedly without showing any adverse effects.

GUT CONTENTS AT DIFFERENT DEVELOPMENTAL STAGES

Jenseen in the year 1967 described the food habits of the green frog, *Rana clamitans* before and after metamorphosis. He made a comparative study between the gut contents and water borne organisms for a period of 7 months and found no changes in the diet till the period of emergence of the fore limbs. However from this stage they did not feed. He also showed that tadpoles consumed food items in relative proportions as they existed in the water. Dickman (1968) on the other hand investigated the effect of grazing by the tadpoles on the structure of a periphyton community in a small Canadian lake. The overall effect of tadpole grazing was to reduce the species diversity of the periphyton triggering a secondary succession.

Heyer (1973) analysed the gut contents of 17 anuran tadpoles and found them to contain mostly Arthropods, Nematodes, Diatoms, filmentous algae and Protozoans besides others. He also classified the feeding habits into 3 distinct types.

Altig and Dearman (1975) studied the percent assimilation (organic material removed from the food during passage through

the gut) and clearence time (time for the food to pass through the gut) of five anuran tadpoles. They also found that tadpoles may shift their feeding emphasis in relation to the abundance of a given food item in their environment because of temperature ontogeny, food quality, population density and ingestion rates. Sabnis and Kolhatkar (1977) studied the food preference of tadpoles of *Rana cyanophylyctis* in India and found them to feed mostly on spirogyra and other zooplanktonic forms. They found variations in dietry components at different growth stages. Sabnis and Kuthe (1978) also studied the food preference of tadpoles of *Bufo melanostictus* at different growth stages. The food preference in general was in the following order Eudorina>Cosmarium>Water mites>Pleurococcus>Diatom>Desmid>Closterium>Ulothrix>Euglena>Navicula>Spirogyra>Seemedesmus. Christian (1982) studied the changes in the food niche during post-metamorphoic ontogeny of the frog *Pseudacris triseriata*.

EXPERIMENTAL WORKS WITH ARTIFICIAL DIET

No comprehensive literature is available on the feeding of anuran tadpoles with controlled diet. More important references in this connection are :

In the year 1941 Briggs described that pure spinach diet produces certain minor abnormalities and kidney stones, and therefore recomended a mixed diet, while Briggs and Davidson (1942) observed growth of *Rana pipiens* tadpoles reared on different diets of boiled spinach and liver food. They found

that spinach alone or in combination with liver food produces stones in the kidneys of all the tadpoles reared on it, while liver food and lettuce food fed seperately or together, produce animals with normal kidneys. Gromko et.al. in the year 1973 showed that reingesting feces (coprophagy) enhanced the growth of *Rana pipiens* tadpoles and that feces deposited second time contained fewer calories/g, than feces which had not been reingested. Mohanty - Hejmadi (1974 b) used leaves of *Amaranthus sp* and egg yolk as food for anuran tadpoles, while Wilbur (1977) used canned chopped spinach for optimal growth of tadpoles. Sabnis and Kuthe (1978) studied the effect of different diets on the growth of tadpoles of *Bufo melanostictus*. They found that tadpoles fed exclusively on spinach, starch and detritus indicated that normal growth occurs when fed on spirogyra and spinach. In the same year Sabnis and Kolhatkar observed that tadpoles of *Rana cyanophlyctis* fed on spirogyra showed maximum increase in their weight.

Shivpal and Niazi (1979) used boiled spinach as food during the laboratory study of *Bufo andersoni* larvae, while Mallick et.al. (1979,80) reported that hatchlings in earthern container depended on algal depositions, phytoplanktons and leaves of hydrilla plants. Dash and Hota (1980) used a mixed food consisting of boiled Amaranthus sp, Boiled egg yolk and cooked minced goat meat as food for the successful laboratory culture of anuran larvae. Semlitsch and Caldwell (1982) on the other hand used only boiled lettuce for rearing tadpoles of *Scaphiopus holbrooki*.

2. LARVAL DENSITY AND GROWTH

In the early 1900's number of workers investigated on the limiting effect of volume of aquatic forms on their growth rate. Crabb (1929) investigated this aspect on pond snails and stated that food, foul media and crowding influenced their growth rate. Adolph in the year 1931, studied the size of the body and the size of the environment in the tadpoles, and found that under optimal conditions growth is very slow between fertilization and hatching. It proceeds with logarithemic increase and then declines in rate. The crowding of many individuals together causes little change in the initial rate of logarithmic increase, but declines much sooner and more severly as the initial density is increased. Lynn and Edelman (1936) studied the correlation between available space and metamorphosis in tadpoles of *Rana sylvatica*. They observed that the onset of metamorphosis is significantly delayed in crowded cultures. Also the percentage of individuals which metamorphose successfully is very closely correlated with the space available per individual.

In the year 1959, Rose described the causes of failure of survival of slowly growing members of a population of *Rana pipiens* tadpoles. She observed that water in which tadpoles or fish have grown inhibits the growth of other individuals of their own kind. Licht (1967) published his work on the intraspecific and interspecific effects of crowding on the growth of tadpoles of

Bufo woodhousii. He found that tadpoles are inhibited in growth when raised in water previously crowded by other larger tadpoles. Brockelman (1969) on the other hand while studying the effects of growth and survivorship of *Bufo americanus* tadpoles and the importance of some predators, found that individual growth variability and mortality were directly related to initial tadpole density but transformation size was inversly related.

Experimental field studies (Brockelman, 1969; Wilbur, 1972, 76 ; Wilbur and Collins, 1973) have also demonstrated that increasing density of caged populations of amphibian larvae decreases both the population that successfully completes metamorphosis and the mean body size at metamorphosis. Wilbur in the year 1972 studied the competition, predation and the structure of the *Ambystoma - Rana sylvatica* community and Gromko et. al. (1973) made an analysis of the crowding effect in *Rana sylvatica* tadpoles and concluded that retardation in growth of tadpoles may be due to a function of effective density, possibly mediated by behavioural or social factors, more importantly than by diffusible products of tadpoles. In the same year, Wilbur and Collins studied the ecological aspects of amphibian metamorphosis and presented growth models for larval amphibians.

De Benedictis (1974) made an experimental field study on the interspecific competition between tadpoles of *Rana pipiens* and *R. sylvatica*. John and Fenster (1975) on the other hand studied the effects of partitions on the growth rate of crowded

Rana pipiens tadpoles. According to them the space is psychological and that stress of crowding is proportional to the frequency of physical encounters between tadpoles. Wilbur (1976) studied the density dependent aspects of metamorphosis in *Ambystoma* and *Rana sylvatica*. He reported that the mean body size at metamorphosis decreases exponentially as the initial density is increased; only fewer individuals are able to obtain sufficient resources to successfully complete metamorphosis. De Benedictis (1977) made a critical review of the meaning and measurement of frequency dependent competition, while Wilbur in the same year studied the effect of density on growth and metamorphosis in *Bufo americanus* tadpoles. He found that survival during the larval period was independent of population density; however the proportion of the population that successfully metamorphosed was a negative exponential function of density. He interpreted the results to be due to effect of density on growth rate of larvae. In high density populations, a few individuals grow at the expanse of small members of the cohort, when they have a lowered probability of metamorphosis. Steinwascher (1978) studied the relative importance of exploitative and interference mechanisms for intraspecific competition among tadpoles of *Rana utricularia*. He interpreted the decreased growth in conditioned water as resulting from chemical interference competition.

Gill also in the same year, presented a model on selection at high population density. According to him several alternative pathways can be taken by a population subject to persistant high

density conditions. The pattern of spatial and temporal predictability in habitat quality dictates whether selection will favour dispersibility, diapause, competitive ability, or tolerance to crowded conditions. The latter may be indistinguishable from evolution of "Allee effects". Dash and Hota (1980) studied the survival, growth rate and metamorphosis of *Rana tigrina* tadpoles. They reported that in laboratory population, survival period was independent of initial population density, but the proportion of the population that successfully completed metamorphosis was a negative exponential function of density. They intrepreted this as the result of slower growth rate in high density populations arising from the competition for food.

Semlitsch and Caldwell (1982) studied the density dependent aspects of growth, metamorphosis and survivorship of *Scaphiopus holbrookii* tadpoles under laboratory conditions. They reported that the mean number of days to metamorphic climax was positively associated with the initial density treatments, while the survival of tadpoles decreased exponentially with initial density. Alford and Crump in the same year investigated the effect of seven environmental parameters in order to document the spatial distribution of three size classes of *Rana utricularia larvae*. They reported that only the largest size class was significantly correlated with percent sand cover.

Nakata et.al. (1982) presented a model for the crowding effect in the growth of tadpoles. They observed that tadpoles in a limited volume grow to divide into 2 groups, a normally growing and a stunted group. This phenomenon was found to be interpreted by a model involving mutual inhibition of growth among individuals.

In 1984, Travis presented an experimental test of a model based on intraspecific competition, for anuran size at metamorphosis, while Sokol (1984) reported on the plasticity in the timing of metamorphosis in tadpoles of *Litoria ewingii*. He found that tadpoles in crowded cultures had a slower growth rate, longer larval periods and smaller size at metamorphosis than tadpoles reared at lower densities. The result of a partition experiment showed that inhibition of development under crowded conditions is mediated by diffusible factors rather than by behavioural interaction. In the same year Slatkin and Anderson presented a simple model of competition among individuals for space. According to them when two individuals attempt to occupy the same area, one or the other dies. Travis and Trexler (1986) studied the interactions among factors affecting growth, development and survival of *Bufo terrestris* tadpoles in experimental populations,

3. TEMPERATURE

TEMPERATURE AND DEVELOPMENT

The effect of temperature on development is a selective factor acting upon life history, mating systems and habitat

selection in amphibians. (Howard, 1978, Berven et.al 1979). Low temperatures causing slower development may limit where a species can breed if temporary aquatic habitats dry up before metamorphosis can occur or if prolonged exposure to predators reduces the number of eggs hatching or larvae metamorphosing. High temperatures may result in developmental abnormilities which like wise reduces hatching success.

The developmental response to temperature variations has been investigated in a number of amphibians since this century. Atlas in the year 1935, described that *Rana pipiens* embryos become increasingly tolerant with age to high temperatures, while Moore (1939,42) correlated the embryonic temperature adaptations with their distribution. However Dushanes and Hutchison (1941) were of the view that genetic differences were responsible for the distribution of a species. In the year 1961 Hubbs and Armstrong studied the development of *Scaphiopus couchi* embryos at various temperatures. At $10^{o}C$ complete mortality was there, while between $16^{o}C$ and $19^{o}C$ most of the embryos hatched in 4 to 6 days. At temperatures ranging from $24^{o}C$ to $28^{o}C$ the embryos were found to hatch in one day itself. Herrid and Kinney(1967) however did not find any significant difference in developmental rates of *Rana sylvatica* at fluctuating or constant temperatures, but Brown in the same year found that in *Scaphiopus hammondii* embryos, temperature tolerance is influenced by the stage of development. Zweifel (1968) made detailed studies on the reproductive biology of anurans inhabiting desert grasslands and arid upland and adjacent

New Mexico. His observations were confined only to embryonic stages as he was of the view that embryos attaining stage 20 in normal conditions will continue to develop normally.

Mc Laren and Cooley (1972) analysed the embryonic temperature adaptations of 14 species of ranid frogs from North America, Europe and Japan, while Kawamura et.al. in the same year studied the time taken for eggs to reach late tadpole stage (forelimb stage) at temperatures ranging from $12^{o}C$ to $34^{o}C$. Their data shows that variations become large at both the upper and lower limits of the temperature scale. Kuramoto (1975) while working on 12 anuran species in Japan, described that embryos of frogs which breed and hatch in summer or warm waters are smaller and consume less O_2 than those which breed in winter or cold waters. Metabolic rate is also higher in warm waters.

Brown in the same year reported that *Ascaphus truei* has narrow temperature tolerance range and slow rate of development and also reported on the embryonic temperature adaptations and distribution of two widely seperated populations of *Hyla regilla* in relation to their environment. He also reviewed temperature adaptations of many anuran species and discussed their geographic and ecological distribution. Justus et.al. (1977) while studying the developmental rates of 2 species of toads (*S.bombifrons* and *S. couchi*) from South West desert, found little variation in developmental rates at temperatures of $18^{o}C$, $20^{o}C$ to $22^{o}C$ and $30^{o}C$, but Sussaman and Belz (1978) described the embryonic stages and timing of *B.orientalius* at $18^{o}C$ and reported an increase in the

standard deviation of time taken to reach a particular stage as development proceeds.

Pawloska-Indyk (1980) studied the development of eggs of *Bombina variegata* between temperature ranges of 12°C to 31°C. He found optimal temperature range to be between 18°C to 28°C , while Michael (1981) investigated the effect of temperature on the rate of development of *Bombina orientalis*. Normal development occured between 20°C to 22.5°C with very little variation in time. However between 18°C large time deviations were seen and at low temperatures below 9°C abnormalities were frequent.

In the year 1983, Travis reported a positive correlation between survival and average size of larvae and interpreted this as the result of size limited predation by insects. Townsend and strewart (1986) studied the effect of temperature on direct development of a terrestrial breeding neotropical frog, *Eleutheradactylus coqui* and found that the range of temperature development was higher than any other aquatic temperate frog except *Ascaphus truei*. They discussed the variations in developmental rate as a consequence of seasonal variation in temperature. Gelder (1987) studied the optimum temperature in egg development of *Rana temporaria*. At 4.5°C and 25.0°C the eggs did not reach stage 15 (early neurula). The optimum temperature range was between 13°C and 18°C.

The work on temperature aspects in India has been very scanty. Das Gupta and Grewal (1968,70) reported that the popula-

tion of *Rana cyanophlyctis* from North India has lower and upper limiting temperatures of 22°C and 31.5°C respectively, but a population from South India has lower limiting temperature of 17°C to 18°C. Roy and Khare (1979) found a wide tolerance range of 5°C to 28°C for *Rana limnocharis* embryos. At temperatures of 5°C mortality was high and abnormalities were also more, where as at high temperatures of 28°C, the embryonic development was fast but was inhibited during post embryonic stages.

THERMAL TOLERANCE AND THERMAL ACCLIMATION

The contribution on thermal tolerance and thermal acclimation started as early as in 1928 when Hathaway was able to show that tadpoles of *Bufo terrestris* had different upper lethal temperatures depending on the temperature of acclimation. Mellanby (1940) also arrived at similar results in *Rana temporaria* and *Salamandra salamandra*, but indicated that the length of exposure to temperatures was an important factor to temperature acclimation. Gosner and Black (1955) found that the thermal tolerance level of *Scaphiopus holbrookii* was 37.5°C. In the same year Mc Farland discovered that the lethal temperatures of the Salamander, *Taricha torosa* when acclimated between 10°C and 30°C were 33.5°C and 36°C respectively. He was of the view that heat resistance of these animals is markedly modified by the length and temperature to which they have been exposed previously.

In 1957 . Volpe while working on the embryonic temperature adaptations of *Bufo valliceps* described that there was a correlation between embryonic temperature adaptation and developmental rate and temperature tolerance and breeding season temperatures, or breeding habits and geographic distribution of the species. Straw (1958) only reported the critical thermal maxima (CTMax) of *Bufo exsul* to be 41°C to 42°C.

More intensive contributions seem to have started in early sixties. Hubbs and Armstrong (1961) studied the minimum developmental temperature tolerance for *Scaphiopus couchi* and *Microhyla olivacea*. Hutchison also in the same year determined the CTMax of 29 species and sub-species of adult salamanders and found lower heat tolerance in larval newts than in adults. Brattstrom and Lawerance (1962) observed that the CTMax or temperature at which coordinated locomotion disappears, increses in relation to increased acclimation temperature in adult frogs and toads and that the effect of acclimation on the CTMax of anurans may be of considerable magnitude. They observed differences upto 5.1°C for individuals of *R.clamitans* and 5.8°C for *S.holbrookii* in animals acclimated at 23°C.

In the year 1965 Brooks and Sassaman studied the CTMax of larval and adult *Eurycea bislineata*. Larvae acclimated at 4°C had a significantly lower CTMax of 33.1°C than those acclimated at 20°C which had a mean CTMax of 34.5°C. Adults acclimated at 4°C had a mean CTMax of 31.6°C, while those acclimated at 20°C had a mean CTMax of 34.6°C. Brown (1967 a) determined the high

temperature tolerance of eggs of *S.hammondii* and found that the upper limiting temperature for normal development in later embryonic stages is 39°C to 40°C. He corrleated these embryonic features with the temperature of the breeding seasons. Again in the same year Brown compared the embryonic temperature adaptations of disjunct allopatric populations of *S.hammondii*. He found that the highest lethal maximum temperature tolerance limit and a faster rate of development than those from the South-West California.

In the year 1968, Heatwole et.al. described the heat tolerances of two species of tropical anurans, *Leptodactylus albilabris* and *Bufo marinus* and found that these levels differ from species to species. Similarly Brattstrom (1968) noted wide variety of habitats, latitudes and altitudes in North and Central America and examined how it was related to their distribution. Dunlap (1968) determined the CTMax of adults of *Pseudacris triseriata* and *Acris crepitans* and found it to be a function of temperature of acclimation . Brown (1969) also showed that the heat resistance of anuran tadpoles can be increased by acclimating to high temperatures and that the heat resistance correlated with the species geographic distribution and breeding habits.

Hutchison and Ferrance (1970) determined the thermal tolerances of *Rana pipiens* acclimated to daily temperature cycles. In the same year Brattstrom determined the CTMax, hot and cold lethals, and rate and range of acclimation for 42 species of anurans from

a wide range of latitudes and altitudes along the East coast of Australia. He found that cryophylic anurans are more northern in distribution, though both Stenothermic and Eurythermic frogs may occur at any latitude. Claussen (1973) determined the thermal relations of the tailed frog *Ascaphus truei* and the pacific tree frog *Hyla regilla*. He found *A. truei* to be stenotopic anuran occuring in cold mountain streams, while *H.regilla* occured in a wide variety of habitats which are thermally diverse.

Cupp (1974) reported a thermal tolerance of 42.5°C for *Bufo woodhousii* tadpoles, while the highest reported is 43.3°C for *Gastrophyrne carolinensis*. Dunson (1977) determined the tolerance to high temperatures and salinity by tadpoles of *R.cancrivora*.

In eighties comparatively few a contributions appeared on the temperature tolerance of amphibians, though they are more intensive. Cupp (1980) observed intraspecific differences in CTMax of tadpoles of *Gastrophryne carolinensis* from different localities and in tadpoles of *B.woodhousii* collected from the same locality at different times. He also determined the CTMax at temperatures of 10°C, 20°C and 30°C during larval development and metamorphosis in 5 salientian species, and observed a correlation between geographic distribution and CTMax and breeding time and CTMax for these species.

TEMPERATURE TOLERANCE DURING DEVELOPMENT

EMBRYONIC STAGES

Since the work of Atlas (1935), who demonstrated that *Rana*

pipiens embryos became increasingly tolerant with age to high temperatures, workers like Moore (1942) studied this factor on certain North American frogs and found that temperature adaptation of embryos correlates well with their distribution. There are a few reports on the short term thermal tolerance of frog embryos. Brown (1967) with the spadefoot toad *S.hammondii* and Herrid and Kinney (1967) with the wood frog, *R.sylvatica* have shown that critical thermal limits vary widely with developmental stages; embryos at cleavage stage are more sensitive to extreme temperatures when compared with gastrula and later stages of development. Zweifel (1968) exhaustively investigated the reproductive biology and embryonic temperature tolerance in 9 anuran species inhabiting desert grasslands and arid uplands and adjacent New Mexico. Mc laren and Cooley (1972) analysed the embryonic temperature adaptation of 14 ranid species from North America, Europe and Japan, while Zweifel (1977) performed a comparative study of short term embryonic thermal tolerances in several frogs from USA. Kuramoto (1978) described that the thermal tolerance of frog embryos was an important function of developmental stages.

LARVAL AND POST-METAMORPHIC STAGES

Hathway (1928) noted that the thermal tolerance of tadpoles of *Bufo americanus* decreased through metamorphosis. The tadpoles had the lowest tolerance in the process of loosing their tails. Brattstrom and Warren (1955) noted that the larval stages of *Hyla regilla* had a higher thermal tolerance than their adults.

However Orr (1955) during the same period found that tadpoles of *Rana pipiens* responded in an appropriate manner, ie. the adults had higher thermal tolerance than larval stages. Herrid and Kinney (1967) reported a decrease in thermal tolerance of *R.sylvatica* tadpoles during the late stages of development and observed a temperature range of $9^{o}C$ to $20^{o}C$, while Licht and Brown also in the same year found the thermal preferrence of the red bellied newt, *Taricha rivularis* to be independent of developmental stage. However Heatwole et.al (1968) reported that thermal tolerance of larval stages of *Bufo marinus* was higher than adults. De Vlaming and Bury (1970) reported that second year tadpoles of tailed frog, *Ascaphus truei* aggregated at warmer temperatures than first year ones. Delson and Whiteford (1973)and Cupp (1974) noted a decrease in thermal tolerance during metamorphic climax of tadpoles. Similar observations have been reported by Hoppe (1978) who found that tadpoles of piedmont and Montane *Pseudacris triseriata* during the stage of hind limbs, had significantly higher CTMax that at the stage with both limbs developed.

Cupp (1980), while studying the temperature tolerance of 5 salientian species found that they were least tolerant to high temperatures at early and late stages in larval development with greatest tolerance at intermediate stages. The postmetamorphic juveniles showed an increase in CTMax, but adults usually had a lower tolerance level than the larvae. Sherman (1980) studied the CTMax of larval, metamorphosing,postmetamorphic (juveniles) and adult toads of *Bufo woodhousii* and found lowest tolerance to

to high temperatures at metamorphic stages. This was followed by a gradual increase in CTMax from juvenile to adult condition, suggesting that the physiological systems underlying thermal tolerance change or mature following metamorphosis untill the adult condition is reached. Dupre et.al. (1982) reported that the preferred temperatures of bull frog tadpoles (*Rana catesbiana*) increased with developmental stage. Floyd (1983) concentrated mainly on the high and low temperature tolerance levels of *B. marinus* and reported that the CTMax increased gradually during the early stages of development, then remained relatively constant through intermediate stages and decreased rapidly as the climax of metamorphosis was approached. Dupre and petranka (1985) studied the preferred temperatures of the tadpoles of 4 amphibian species and found that the mean temperature preferrence of all the species increased with developmental stage, reaching a peak shortly before metamorphic climax.

THERMAL TOLERANCE : DAILY RHYTHMS

Studies on the daily rhythms of thermal tolerance under laboratory conditions is very scanty.

Adults

The first report of daily rhythms in thermal tolerance was given by Kosh and Hutchison (1968) in the eastern painted turtles, *Chrysemys picta*. They reported that turtles maintained at constant temperatures of $10^{o}C$ and $20^{o}C$, at 8 hr and 16 hr photoperiods

are more sensitive to temperature at certain hours. Dunlap (1969) observed some daily variations in adults of *Acris crepitans,* while Mahoney and Hutchison (1969) found daily vatiations in temperature tolerance for *R. pipiens and Hyla labialis.* Johnson (1971b, 1972a,b) observed distinct temporal patterns for *Litoria caerula, Bufo marinus and L.gracilenta.* He considered the temporal pattern to be adaptive in that thermal tolerances are highest when environmental temperatures are correspondingly high. Hutchison and Spriesterbach (1986) determined the diel and seasonal cycles of activity and behavioural thermoregulation in the salamander *Necturus maculosus.*

Tadpoles

There are reports on the daily cycles of distribution and activity of tadpoles under natural conditions since early 1900's. Bragg (1946), Richmond (1947) and Carpenter (1953) discussed the aggregative behaviour in tadpoles. Similar observations were made by Mullally (1953) for *Bufo canorus;* by Brattstrom and Warren (1955) for *Hyia regilla;* by Brattstrom (1962) for *R. boylei, B.boreas* and *H.crucifer;* and by Tevis (1966) for *Bufo species.*

Beiswenger and Test (1967) found that the tadpoles of *Bufo-terrestris* and *B. americanus* spend the night scattered in deeper parts of a pond and moved in the morning into the shallow areas where they aggregated during the day. They analysed that the

response of larvae to temperature gradients was the major cause of this distributional pattern. Anderson (1968) found that the larvae of *Ambystoma macrodactylum* came into the warmer areas of the pond which had more light than adjacent areas, while Ashby (1969) and De Vlaming and Bury (1970) reported similar observations for *Rana temporaria* and *Ascaphus truei* tadpoles respectively.

Adler (1970) noted that light plays an important role in the regulation of behaviour rhythms of amphibians. Beiswenger (1975, 1977) described the aggregations of tadpoles of *Bufo americanus* in relation to light and temperature factors and in 1978 reported that tadpoles of *Bufo boreas* and *Bufo hemiophrys* seek out and occupy warm regions of thermally stratified habitats.

Although there are a few reports on temperature preference of tadpoles under laboratory conditions (see De Vlaming and Bury, (1970; Beiswenger, 1978), there is only one report on the daily rhythm of thermal tolerance. Willhite and Cupp (1982) reported on the daily rhythms of thermal tolerance for the tadpoles of *Rana clamitans*. They observed a distinct rhythm of Critical Thermal Maxima (CTMax) variation over 24 hr period. This rhythm appeared to anticipate daily changes in pond temperature.

MATERIAL AND METHODS

3.1 COLLECTION OF TADPOLES

The tadpoles of *Rana limnocharis* were collected at different developmental stages from a natural habitat at Smit and maintained in an aquaria in the laboratory. These tadpoles were used for gut content analysis and experiments on critical thermal maxima (CTMax) and critical thermal minima (CTMin).

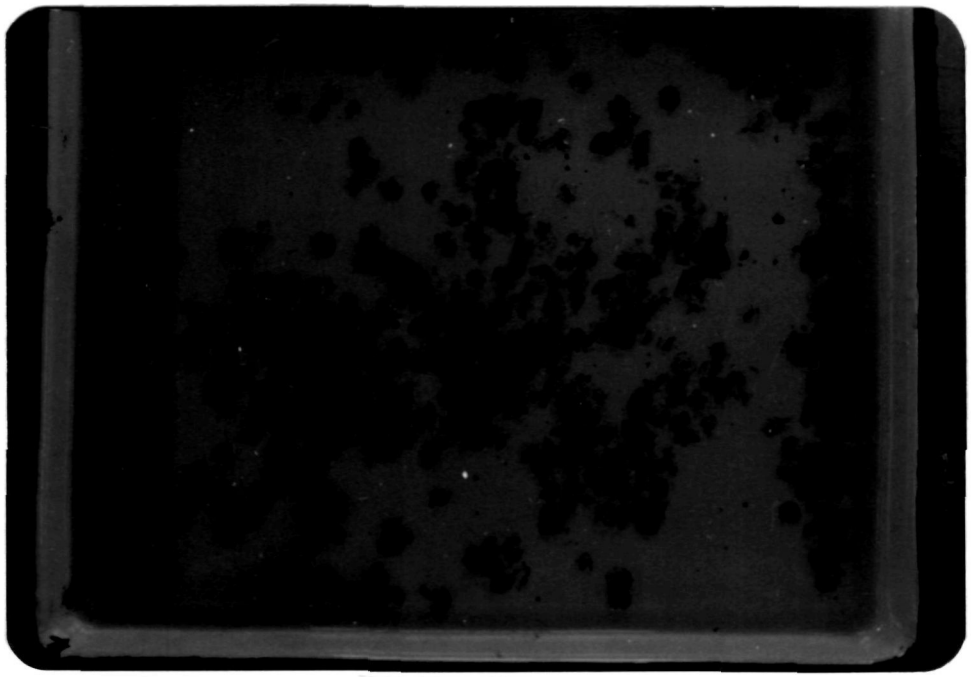
3.2 COLLECTION OF SPAWNS

The spawns of *Rana limnocharis* were collected during the breeding period (April-August) in the early hours of the day from temporary pools from a grassy field at Smit and kept in enamel trays in the laboratory (Plate 3.1a).

3.3 HATCHING AND MAINTENANCE OF TADPOLES

The tadpoles hatched from the spawns were used for analysing the effect of artificial food on development, density related experiments, effect of culture medium on development and the effect of different temperatures on development (Plate 3.1b).

The tadpoles were maintained in unconditioned tap water in large plastic tubs. As the water is collected in a reservoir directly from a spring and supplied in our laboratories without chlorine treatment, we did not treat it with sodium thio-sulphate as suggested by Nace and Richards (1972). The tadpoles were fed



A

B

PLATE 3.1 A - SPAWNS OF *Rana limnocharis*

B - NEWLY HATCHED TADPOLES OF *Rana limnocharis*

on a diet of boiled cabbage and water was changed twice a week. Fresh food was added every time the water was changed. Every day the trays and bowls were checked twice to remove the dead individuals. A steep sand base and stones etc. were provided on one side of each tray/bowl during later stages of development to provide a proper substrate for successful completion of metamorphosis. The experimental set up is shown in Plates 3.2a and 3.2b

3.4 PROCEDURE FOR GROWTH ANALYSIS

In the present study, two important parameters have been used for analysis of the growth of tadpoles i) Body size, ii) Body weight. Gromko et al. (1973) and Semlitsch and Caldwell (1982) used tadpole size (cube root volume) as an estimate of larval growth, where as certain notable workers such as Adolph (1931), Licht (1967), Dash and Hota (1980), Mishra and Dash (1984), Travis (1984) and Petranka (1984) have used body weight as an estimate of larval growth. Smith (1983) on the other hand used length as a growth parameter. In the present study we have also followed this criteria and larval growth has been estimated in terms of increase in body weight with time. Morphometric measurements such as body length and tail length have also been recorded (Fig. 3.1). Weights were taken either singly after blotting with blotting paper or in groups in pre-weighed beakers containing some water. The mean larval weight was calculated from replicates. All weights were taken on a K. Roy Chemical balance (precision 0.001g)

A

B

PLATE 3.2 A & B - EXPERIMENTAL SET UP.

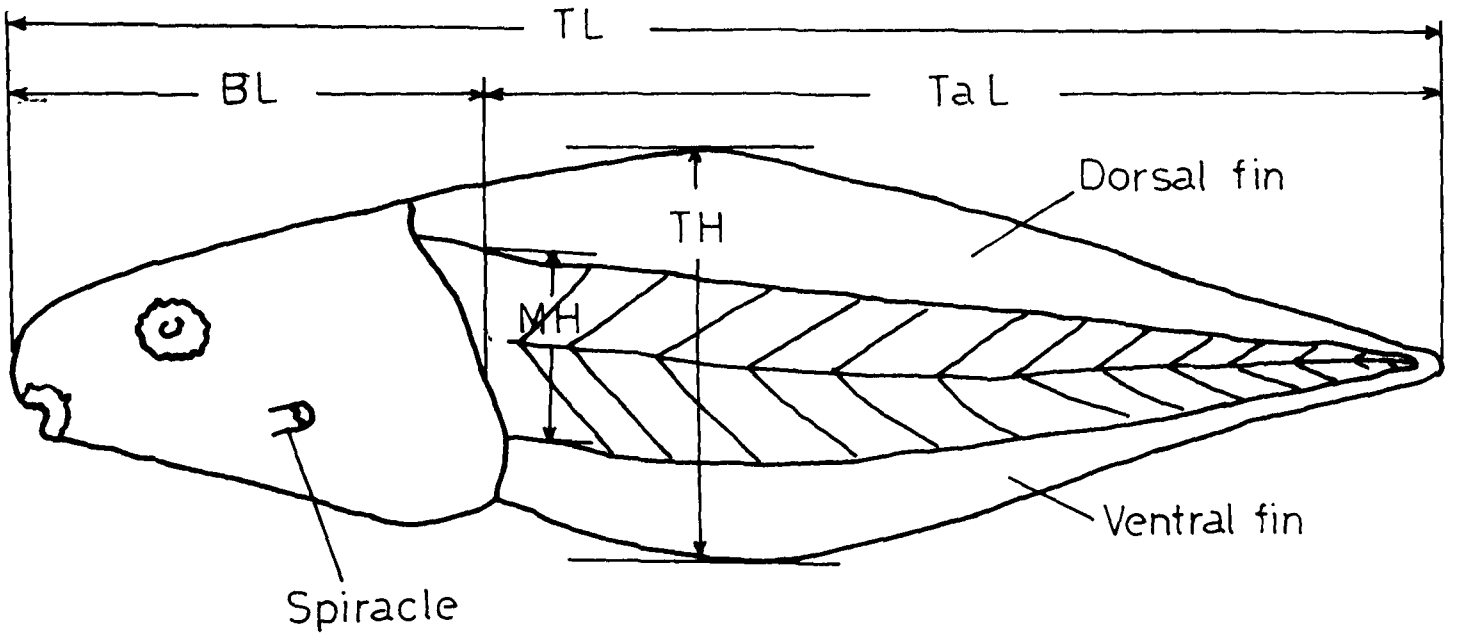


FIG. .3.1 LATERAL VIEW OF TADPOLE : PARAMETERS FOR MORPHOMETRIC MEASUREMENTS.

(TL - TOTAL LENGTH (TIP OF SNOUT TO TIP OF TAIL)
BL - BODY LENGTH (TIP OF SNOUT TO JUNCTION OF BODY)
TaL- TAIL LENGTH (JUNCTION OF BODY TO TIP OF TAIL)
TH - TAIL HEIGHT

or on a Sartorius balance (precision 0.01 g). Every day 5 randomly selected individuals were examined under a dissecting binocular microscope for a detailed study and staging of the tadpoles.

3.5 NORMAL DEVELOPMENT OF TADPOLES (CONTROL)

In order to study the normal development of tadpoles from hatching to completion of metamorphosis, 10 tadpoles(2 d old) were reared in enamel trays of size 37 x 44 x 5 cm each containing 1.5 ℓ of unconditioned tap water. There were 3 replicates for the experiment. A fixed ration of 1.2 g/tray/2 days of boiled cabbage was used as food for the tadpoles. Morphometric measurements such as total length, body length and tail length were taken as illustrated in Fig. 3.1. Every day 5 randomly selected tadpoles were examined under a dissecting binocular microscope and their stage of development was noted. The tadpoles were staged according to Roy & Khare (1978).

3.6 PROCEDURE FOR EXPERIMENTS IN RELATION TO FOOD

3.6.1 Analysis of Gut contents of Tadpoles

For analysis of gut contents, tadpoles at hind limb stages (stage 26-27) were collected directly from the natural habitat at Smit and preserved in 4% formalin. After fixing, the tadpoles were dissected and the guts were removed. Gut contents were analysed by taking 10.0 mm of the fore gut and 10.0 mm of

the hind gut. Each of the segments were mixed with 1.0 ml of distilled water and a drop of lugol's iodine was added. The food items were analysed either under a dissecting binocular microscope or in a Sedgwicks rafter under a compound microscope. For the identification of the food items we followed Edmonson (1959).

3.6.2 Analysis of gut contents at different developmental stages : in relation to development of mouth parts

In order to study the food preferences during development of tadpoles they were selected at stages 21 to 32 and preserved in 4% formalin. The procedure for identification of food items at different stages were similar to the above experiment. At each stage morphology of the mouth parts was also studied. The size of food items consumed and their relationship with the size and shape of mouth and mouth parts during development was also investigated.

3.6.3 Analysis of Development of Tadpoles with Artificial Diet

i) Quality

In order to determine the effect of food quality on growth and metamorphosis, six different types of food items were used 1) Boiled cabbage, 2) Boiled lettuce, 3) Boiled egg yolk, 4) Cooked goat meat, 5) Ripe banana, 6) Mixed food (combination of all the food items).

The experiments were started 2 days after hatching (stage 22, average body length 5.75 mm, average weight 0.025 g). The tadpoles were randomly divided into 7 sets of 15 tadpoles each. Each set of 15 tadpoles was reared in 3(replicates) seperate bowls (d = 20 cm) each containing 1 ℓ of unconditioned tap water and with 5 tadpoles. A constant food ration of 0.6 g/bowl was given every two days during the experiment. All the six experiments with different food items were conducted simultaneously.

ii) Quantity

For this experiment 2 day old tadpoles (average body length 5.75 mm, average weight 0.025 g) were randomly divided into 6 sets of 15 tadpoles each. The 15 tadpoles were reared in 3 (replicates) seperate bowls (d = 20 cm) each containing 1 ℓ of unconditioned tap water and with 5 tadpoles. Six different food quantities of 0.1 g, 0.2 g, 0.4 g, 0.6 g, 0.8 g, and 1.0 g/ bowl/2 days were used as food for the tadpoles.

3.7 PROCEDURE FOR EXPERIMENTS IN RELATION TO DENSITY

3.7.1 Tadpoles reared in isolation

For this experiment, 20 tadpoles (2 days old) were reared individually in plastic bowls, each containing 200 ml of unconditioned tap water. A few seperate trays with 10 tadpoles each were also maintained as a stock. A fixed ration of 0.2 g boiled cabbage/bowl was used as food for the tadpoles.

3.7.2 Tadpoles Reared at Different Densities

For analysing the effect of density on the growth and metamorphosis, 2 d old tadpoles were randomly selected into the following densities of 4, 8, 16, 32, 64, 128 and 256. The tadpoles were reared in enamel trays of size 37 x 44 x 5 cm each containing 2.5 ℓ of unconditioned tap water and each density treatment was carried out in three replicates. A fixed ration of 1.0 g of boiled cabbage was given in each tray for the first two weeks and then it was increased to 2.0 g for the remaining experiment.

3.7.3 Effect of Culture Medium on the Development of Tadpoles

For this experiment two sets of tadpoles (n = 20) were used. In one set the water was not changed throughout the experiment except that fresh water was regularly added to replace the water lost by evaporation. In the second set water was regularly changed twice a week. A fixed ration of 0.6 g/5 tadpoles/ 2 days of boiled cabbage was used as food for the tadpoles.

3.8 PROCEDURE FOR EXPERIMENTS IN RELATION TO TEMPERATURE

3.8.1 Effect of Temperature on the Development of Tadpoles

To study the effect of temperature on growth and metamorphosis two days old tadpoles were randomly divided into 3 sets of 20 tadpoles each. Two sets were reared in B.O.D. incubators at constant temperatures of 10°C and 32°C respectively. The

third set was reared at room temperature ($\bar{x}$ 23°C) as a control set. Each set of 20 tadpoles were reared in 2 seperate trays (size 37 x 44 x 5 cm) each containing 1.5 ℓ of unconditioned tap water. A fixed ration of 1.2 g/tray/2 days of boiled cabbage was used as food for the tadpoles.

3.8.2 Estimation of Thermal Tolerance Levels

In order to determine the thermal tolerance levels tadpoles of stages 26-27 (hind limb stages) were collected directly from their habitat at Smit and brought to the laboratory. All the experimental tests were performed within 3 hr of collection in order to minimize the effect of time factor.

i) Critical Thermal Maxima (CTMax)

The critical thermal maxima (CTMax) was determined as per the technique of Hutchison (1961), using the definition of Lowe and Vance (1955) as 'The arithmetic mean of the collective points at which the locomotory activity becomes disorganised and the animal loses its ability to escape from conditions that will promptly lead to its death.' This technique has also been followed by Cupp (1980). The details of the technique are following.

Each tadpole was placed in 100 ml of water in a 250 ml beaker (WT 22.5°C). The test beaker was immersed in a trough filled with 200 ml water which served as a water bath. The water in the

trough was heated over a tripod with a bunsen burner and water was continuosly stirred to maintain uniform temperature. An aerator was used for aeration of water during the experiment. The water temperature was raised at the rate of 1°C/min taking care that there should be no lag between ambient temperature and deep body temperature. In all there were 12 replicates of the experiment. The tadpoles showed uneasy and fast movements at higher temperatures and at the CTMax end point they did not respond to mechanical stimulation with forceps. These temperatures were recorded. The procedure for determining the CTMax of juveniles was similar to that of tadpoles (Fig. 3.2).

ii) Critical Thermal Minima (CTMin)

The CTMin was determined by cooling the water. Each tadpole (stage 26-27) was placed in 50 ml of water in a 100 ml beaker which was immersed in a 250 ml beaker containing 1000 ml of water. The larger beaker was placed in a plastic trough containing ice cubes. The smaller beaker was moved in and out of the ice pack such that the water dropped at the rate of 1°C/min. The criteria for CTMin determination was similar to that of CTMax test.

3.8.3. Analysis of the Effect of Thermal Acclimation on CTMax

In order to study the effect of thermal acclimation on CTMax the tadpoles were collected at stages 26-27 directly from the field and brought to the laboratory. The tadpoles were

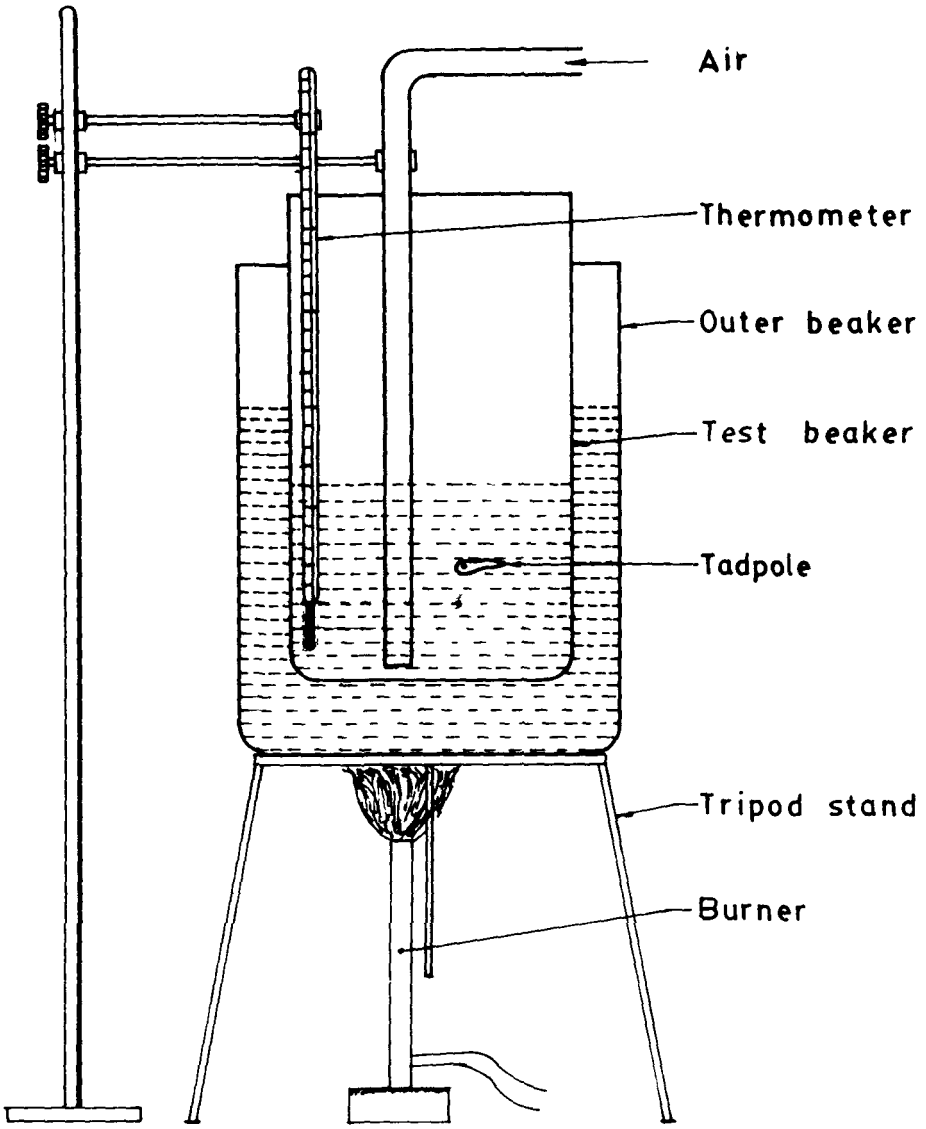


FIG. 3.2 EXPERIMENTAL SET UP FOR DETERMINATION OF CRITICAL THERMAL MAXIMA (CTMax)

randomly seperated into 3 groups of 12 tadpoles each. Two groups were acclimated at 10°C and 35°C respectively in B.O.D. incubators for a period of 4 days during which they were not fed. The third group of tadpoles were maintained at room temperature of 23°C. On the day of determination of CTMax, the tadpoles were removed from the B.O.D. incubators and allowed a 30 min period of acclimation at room temperature in order to avoid thermal shock. The method for the determination of CTMax was similar as in experiment 3.8.2. i.

3.8.4 Analysis of CTMax at Different Developmental Stages

i) Embryonic Stages

For this experiment 4 different embryonic stages namely stage 4, stage 11, stage 17, and stage 22 were used. Ten embryos of each stage were put in beakers containing 500 ml of water and bathed in water baths maintained at three different temperatures of 40°C, 42°C, and 43°C. They were exposed to constant temperatures for a period of 2 hr after which the beakers were allowed to cool down to room temperature. As the water temperature of the beakers rose, the embryos moved upwords to the surface of the water as numerous small bubbles formed on the outer surface of the jelly envelops. These bubles were removed by shaking the eggs gently. After 24 hr of the experiment the number of dead and normal embryos were calculated. Critical temperatures at which half of the embryos died were determined from two temperature values which gave the mortalities around 50%.

ii) Larval Stages

For determination of the CTMax during different stages of larval development, the tadpoles at stages ranging from 23 to 32 and juveniles of South-Vent length ranging from 18 mm to 30 mm were collected directly from the field. The CTMax of larval, metamorphosing and post-metamorphoic stages were determined by the method described in experiment 3.8.2. i. All the experimental tests were performed within 3 hr of collection to minimize the effect of time factor. In all there were 12 replicates of the experiment at each stage of development.

For determination of the CTMin during different stages of development, the tadpoles at stages ranging from 23 to 32 and juveniles of SV length ranging from 18 mm to 30 mm were collected directly from the field. The CTMin were determined by the method described in experiment 3.8.2. ii. All the experimental tests were performed within 3 hr of collection of minimize the effect of time factor. In all there were 12 replicates of the experiment at each stage of development.

3.8.5 Analysis of Daily Rhythms of CTMax

For determination of daily rhythms of CTMax also the tadpoles were collected at stages 26-27 directly from the field and brought to the laboratory. All the tadpoles were acclimated at 24°C ± 1°C in B.O.D. incubators for a period of 4 days during

which they were not fed. A constant photoperiod of LD 12 : 12 centered at 1200 hr was maintained. The CTMax determinations were made at 3 hr intervals begining from 0500 hr. The procedure used for CTMax determination was same as in experiment 3.8.2. i. The changes in atmospheric temperatures during the day time at the study site were also noted.

3.9 TERMINATION OF EXPERIMENTS

The appeareance of the fore limbs was taken to be the beginning of metamorphic climax and full resorption of the tail was taken to be the completion of metamorphosis. Once the tail was resorbed the froglets were removed and released into the ponds.

3.10 STATISTICAL ANALYSIS

For statistical analysis of the experimental data, mean, range, standard deviation (SD), standard error (SE) were calculated and they were subjected to student's t test and variance test (Sokol and Rohlf, 1969).

EXPERIMENTS AND RESULTS

4.1 STUDY SITE : LOCATION AND CLIMATE

Meghalaya, which means the abode of clouds, is popularly described as "The Scotland of the East". And Shillong, its capital, has its own charm distinct from other hill stations. Located at an altitude of 1515 m. a.s.ℓ. and at an intersection of 25°5'N latitude and 91°9'E longitude (Fig. 4.1), Shillong presents a beautiful natural scenery dotted with a number of waterfalls, brooks, pine grooves and gardens; even the mute monoliths do not seem to look lifeless. The flora are peculiar to this region. Smit, the place of collection is located at an altitude of 1070m. a.s.ℓ., 15 Km away from the Deputy Commissioner's Office at Shillong.

Location and physiographic factors have greatly influenced the climate of this region. The climate of this region is controlled by seasonal winds of south west monsoon and the North-East winter winds. The state of Maghalaya has four seasons 1) Spring (March-April), 2) Summer (May-September), 3) Autumn (October-November), 4) Winter (December-February).

During the summer months of April and early May strong winds blow and atmospheric temperature reaches maximum. The months from May to September represent the wet season, with June and July experiencing heavy showers, while occassional showers may occur during March and November as well.

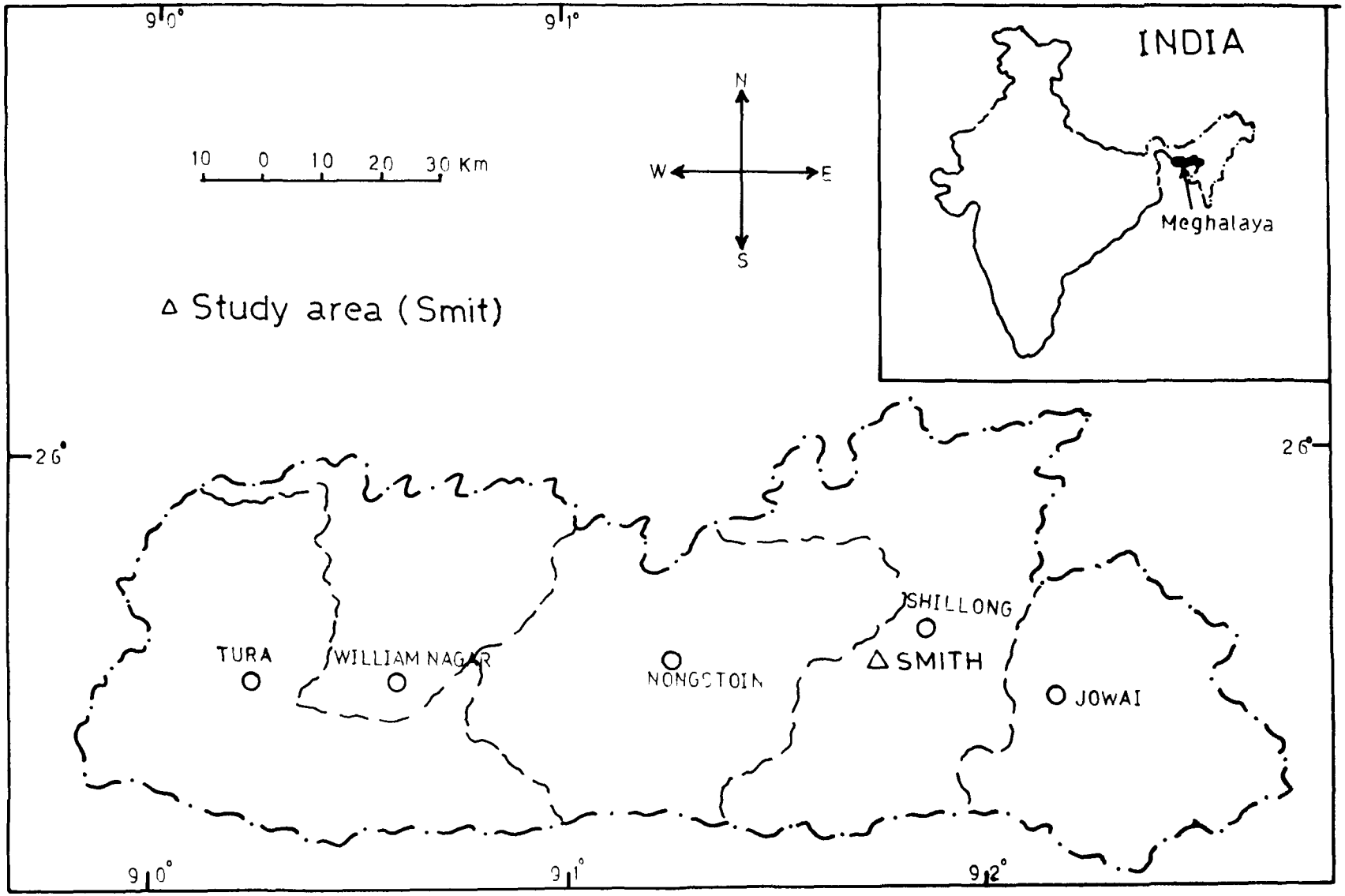


FIG. 4.1 MAP OF MEGHALAYA AND THE STUDY SITE (SMIT).

During the Autumn months of October and November, there is a retreat of monsoon and a fall in the atmospheric temperature.

The cold winter season lasts from December to February and is characterised by low temperature and negligible rainfall.

In general, the state has a sub-tropical climate. During summer, day temperatures may sometimes reach a maximum of 29°C and during winters the night temperature may be as low as 1.2°C, marked by appearance of ground frost at night and early mornings for most of the winter. There is no snowfall in this region. The most interesting feature of this region is very high rainfall, with an average of 2,500 - 3,000 mm/annum; the maximum annual average of 12,000 mm being recorded nearby Cherrapunjee and Mawsynram platforms, the worlds rainiest spots.

Smit, is a small platue surrounded by hills. The area is predominantely a grassy field with plenty of aquatic vegetation growing during the rainy season. The spot is an uncultivated land, although there is an extensive cultivation on the neighbouring hill slopes (Plates 4.1A and 4.1B). During winter months of December to February there is almost no rain in this area and the ponds and pools dry up. The water accumulates in these ponds and pools only after heavy showers in the rainy season from May to September.

A

B

PLATE 4.1

A - GENERAL VIEW OF COLLECTION SITE OF *Rana limnocharis* TADPOLES - SMIT.

B - CLOSE UP VIEW OF COLLECTION SITE - SMIT.

Various characteristic features of the study site have been presented in table 4.1. The size of the ponds and pools ranges from 0.5 to 1.5 m^2. The average light intensity is 18.0 lu. while the average atmospheric and water temperatures are 22.6°C and 22.4°C respectively. The average relative humidity is 85% and the average rainfall is 383.3 mm. The average water pH of the study site is 6.67.

The major vegetation present in the ponds and pools include grasses like *Paspalam Sp., Ganphalium leuto-album, Oxalis corniculata, Oxalis latifolia, Polygonum chinensis, Centella asiatica, Trifolium repens,*and *Fimbristyles Sp.* The major organisms present in the habitat include insect larvae, water beetles, water spiders and water leeches.

METEROLOGICAL DATA

The average monthly records of maximum and minimum temperature, humidity and rainfall were obtained from the local meterological station. These have been illustrated in Fig. 4.2.

A. Atmospheric Temperature

Average monthly maximum and minimum atmospheric temperatures are illustrated in Fig.4.2. The average maximum temperature ranged from 14.8°C in February 1985 to 22.8°C in August 1985 and from 14.7°C in January 1986 to 22.9°C in June 1986.

The average minimum temperature ranged from 1.7°C in January to 16.7°C in August 1985 and from 1.2°C in January 1986 to 16.4°C in July 1986.

B) Relative Humidity

The average monthly relative humidity are illustrated in Fig. 4.2. During 1985, the lowest average relative humidity was 61.5% in March and maximum was 88% in July, while in 1986, the lowest was 50% in March and maximum was 91% in July.

C) Rainfall

The daily records of rainfall were totaled for each month and have been illustrated in Fig. 4.2. During 1985, the month of November had minimum rainfall of 0.6 mm, while the month of July experienced the highest rainfall of 582.8 mm. But during 1986 December recorded the lowest rainfall of 0.80 mm, while July experienced a rainfall of 279.0 mm and September had highest rainfall of 444.6 mm.

Table 4.1 Characteristic features of Study Site - Smit

Altitude	1707 m.a.s.l.
Area of collection site	2500 m^2
Habitat	Temporary pools/ponds(5.0-8.0m^2)
Average light intensity	18.0 Lux 10^3
Average water temperature	22.4^oC
Average atmospheric temperature	22.6^oC
Average Rainfall	383.3 mm
Average water pH	6.67
Major aquatic plants in the habitat	Grasses like *Paspalam species Gnaphalium leuto-album, Oxalis corniculata, Oxalis latifolia, Polygonum chinensis, Centella asiatica, Trifolium repens and Fimbristyles spp.*
Major aquatic organisms in the habitat	Insect larvae, water beetles, water leeches.

* Observations during month of June 1985.

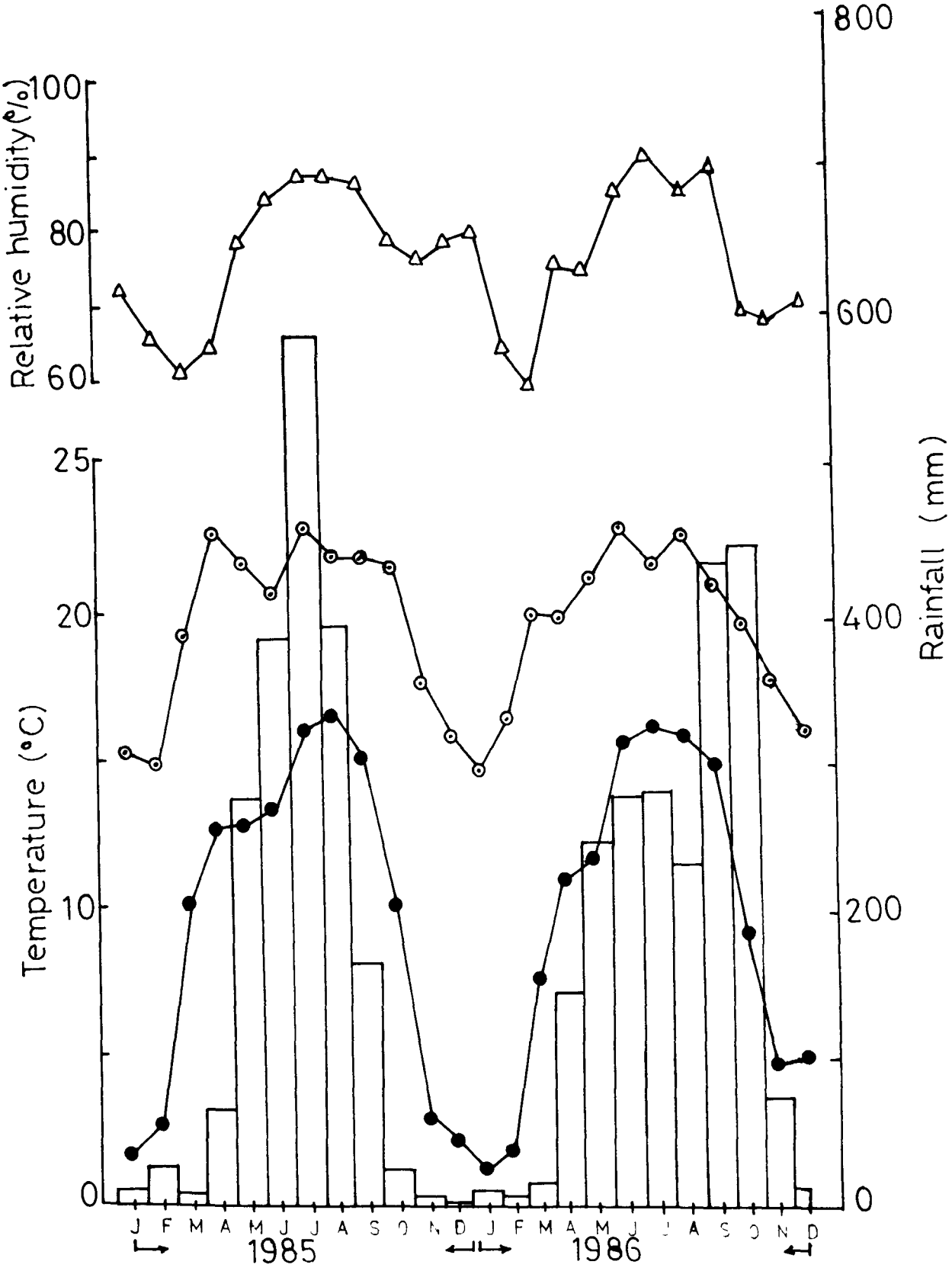


FIG. 4.2 METEROLOGICAL DATA OF MEGHALAYA FOR 1985-1986. (RAINFALL DATA ARE MONTHLY TOTAL; OTHERS ARE MONTHLY AVERAGE).

4.2 BIOLOGY OF *Rana limnocharis*

4.2.1. Classification and Distribution

Phylum	:	Chordata
Subphylum	:	Gnathostomata
Class	:	Amphibia
Order	:	Anura
Sub order	:	Diplasiocoela
Family	:	Ranidae
Sub family	:	Raniane
Genus	:	*Rana* Linnaeus
Species	:	*limnocharis*, Weigmann

Rana limnocharis has a wide-spread distribution. Its distribution extends in Eastern Asia, from Japan and China to India, Ceylon, Malayan Peninsular, Philippines, Borneo, Lombok, Sikkim, Nepal, Thailand, Formosa, Korea, Southern China, Burma, Thailand, Kampuchea, Laos, Vietnam and from Pakistan. Pillai and Chanda (1976) compiled information that it is distributed in Assam, Meghalaya and Arunachal Pradesh.(Fig.4.3) There are reports of its occurance in Tripura also. (see, Kiyesatuo, 1986).

4.2.2. Habit and Habitat

1) Nocturnal as well as diurnal.

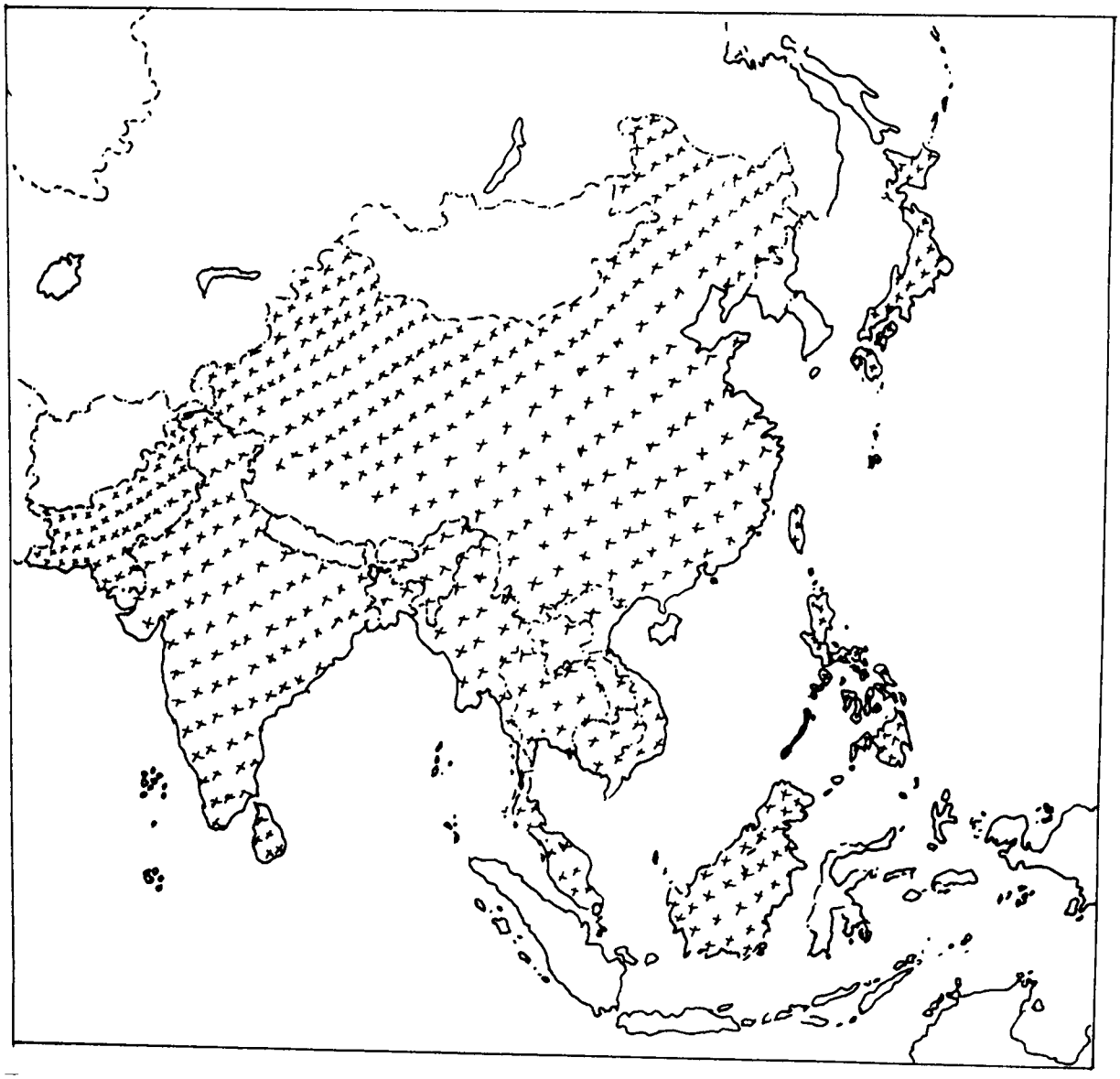


FIG. 4.3 DISTRIBUTIONAL PATTERN OF *Rana limnocharis*.

2) Inhabit paddy fields, eutropic ponds, marshy places and near rivers and pools.

3) Hibernate under the soil or stones during winters.

4) Breed in temporary ponds, pools and paddy fields in summer months of April to August.

4.2.3. Annual Breeding Cycle and Life Cycle

i) Annual Breeding Cycle

The annual life cycle of *Rana limnocharis* has been illustrated in Fig. 4.4. It can be divided into 4 phases namely 1) Hibernating phase, 2) Pre-breeding phase, 3) Breeding phase, 4) Post-breeding phase.

Hibernating Phase

The frogs undergo a period of hibernation during the cold winter months from November to middle or late March. The average minimum and maximum temperatures recorded during November are 3.0°C and 17.8°C; during December 3.1°C and 16.0°C; during January 1.2°C and 14.7°C; and during February are 2.7°C and 14.8°C. The rainfall and average relative humidity during these periods are 0.6 mm and 76.5%; 4.0 mm and 79.0%;10.0 mm and 80.5%; and 24.0 mm and 66.0% respectively. During the hibernating period the frogs usually burrow themselves in the mud or may hide themselves underneath stones.

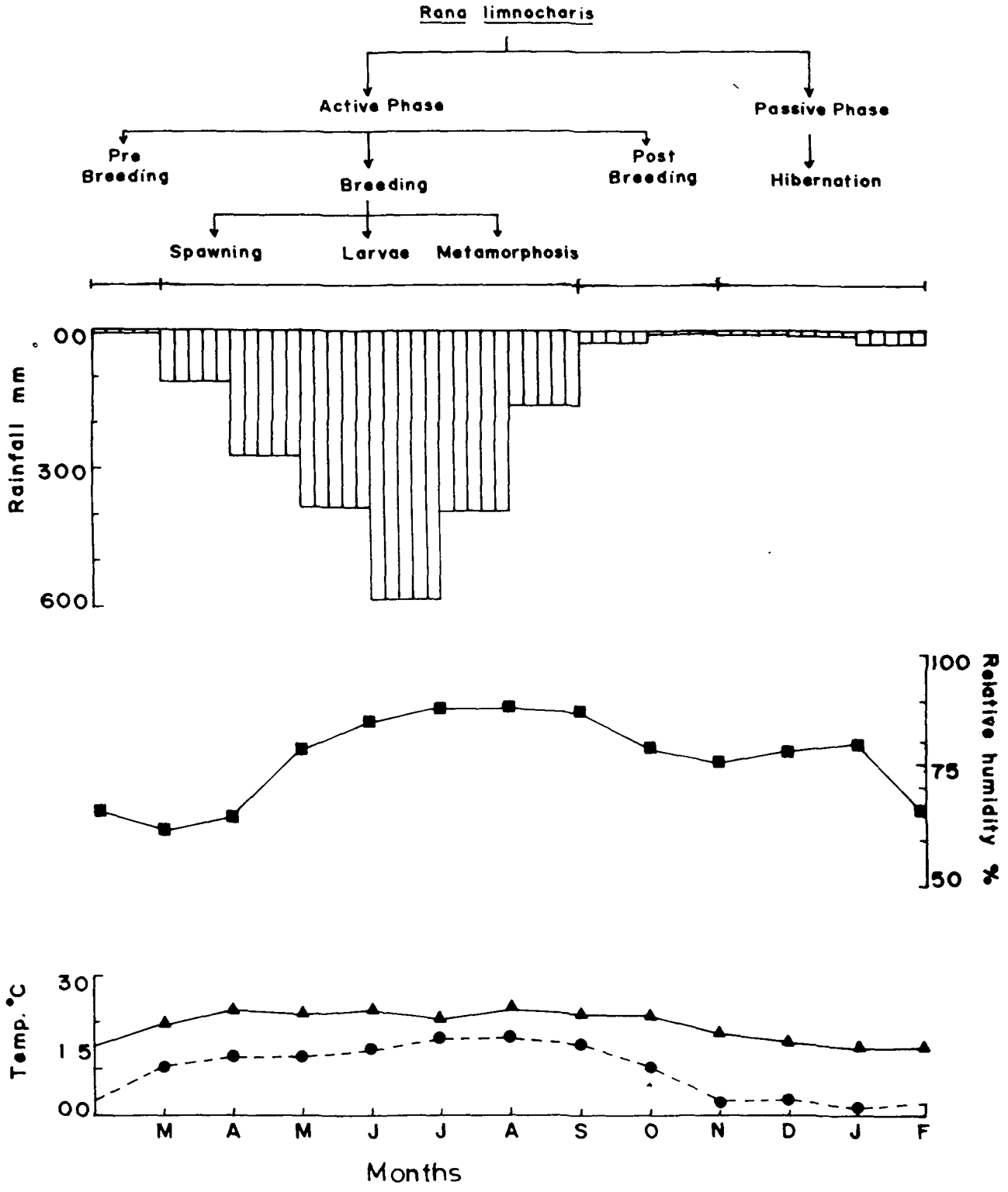


FIG. 4.4 ANNUAL BREEDING CYCLE OF *Rana limnocharis* IN RELATION TO ENVIRONMENTAL FACTORS.

Pre-breeding Phase

Frogs can be seen from middle or late March when there is slight increase in average minimum and maximum atmospheric temperatures to 10.2°C and 19.3°C. The rainfall and average relative humidity during this period are 9.3 mm and 61.5% respectively. With further increase in rainfall the frogs start spawning. This brief period between which the frogs appear and start spawning is referred to as pre-breeding period.

Breeding Phase

The frogs start feeding actively and show amplexing behaviour from early April when the average minimum and maximum temperatures increase to 12.6°C and 22.6°C respectively. During this period the rainfall increasessharply to 114.0 mm, while average relative humidity increases to 64.3%. A large number of spawns are seen attached to aquatic grasses and other vegetations in temporary ponds, pools, ditches and slow flowing streams, while early larval stages are seen from the middle of May when the average atmospheric temperature ranges from 12.7°C to 21.6°C. The rainfall further increases to 273.8 mm, while the average relative humidity is 79.0%. The average atmospheric temperature ranges from 13.3°C to 22.6°C in June; from 16.1°C to 20.6°C in July; and from 16.7°C to 22.8°C in August. The rainfall and average relative humidity during these periods are

383.3 mm and 85.0%; 587.8 mm and 88.0%; and 392.4 mm and 88.0% respectively.

The active breeding period lasts till the end of August. Metamorphosed froglets are seen from early June. Those tadpoles which are hatched from spawns laid during early May, metamorphose by early June, while those laid during late June or July, metamorphose by July or August. This period of active feeding and breeding is referred to as the breeding period.

Post-breeding Period

From September onwards when the average minimum and maximum atmospheric temperatures reduce to 15.2°C and 21.8°C respectively, the number of frogs seen reduces, untill after October frogs are rarely seen. The rainfall also reduces to 162.8 mm and average relative humidity to 87.5%. This short period between September and October is referred to as the post breeding period.

ii) Life Cycle

The life cycle of *Rana limnocharis* has four stages 1) Adults, 2) Eggs , 3) Larvae and 4) Juveniles.

1) Adults

Morphological Characteristics

i) Moderately small frog

ii) Head as long as broad.

iii) Snout pointed or rounded, projecting beyond the mouth.

iv) Nostrils present between the eyes and tip of mouth and nearer to the snout.

v) Skin of dorsal surface greyish brown with or without mid dorsal line or with or without 2-3 reddish spots. Skin coarsly granular with broken ridges.

vi) Skin fold is present above the tympanum and behind the eyes.

vii) Belly smooth and yellowish white

viii) Fingers free, toes webbed.

ix) Fingers and toes with bluntly pointed tips and 1st finger longer than 2nd.

Distinguishing characteristics of Males and Females

Males

i) Small and light in weight, having a streamlined body.

ii) Snout-Vent length ranges from 3.0 cm to 3.9 cm ($\bar{x}$ 3.52).

iii) Weight ranges from 3.3 g to 4.4 g ($\bar{x}$ 4.02 g).

iv) Thumb pads present and are very prominent during the breeding season.

v) Black markings present on vocal sacs below the throat. The vocal sacs are used for producing croaking sound during the breeding season.

Females

i) Snout-Vent length ranges from 3.7 to 5.2 cm ($\bar{x}$ 4.62).

ii) Weight ranges from 7.8 g to 13.5 g ($\bar{x}$ 11.54 g).

iii) Larger and heavier than males.

iv) Abdomen swollen due to presence of ovaries.

v) Skin of groins transluent so that mature ova can be seen through it.

2) Eggs

The amplexing females deposite spawns usually during the early morning hours. As soon as the eggs are laid, the jelly swells up (0.09 mm) and the spawns are seen attached to the surrounding grasses and other aquatic plants. The number of eggs in each spawn varies from 400-800 eggs.

Fertilized eggs are darker on the animal pole than that on the vegetal pole. The average diameter of each egg is 1.5 mm.

3) Hatching and Larval Stages

The embryonic period lasts for 4 to 5 days. The larvae after hatching attach themselves to water plants or rest at the bottom of the ponds. The period from hatching till complete resorption of tail is designated as larval period and is usually completed in about 32 days. The larvae metamorphose and give rise to Juveniles.

4) Juveniles

Newly metamorphosed juveniles live on the banks of ponds or edges of other water bodies and jump into the water when disturbed. The juveniles grow in size and are later transformed into the adults.

4.2.4 Normal Developmental Pattern of Tadpoles

Though developmental process is a continuous and dynamic process, for the sake of convenience the larval stages can be broadly divided into three distinct periods or phases (Etkin,1964) as i) Premetamorphic phase. ii) Pro-metamorphic phase. iii) Metamorphic climax. Various morphometic measurements recorded, during development have been tabulated in a Table 4.2. and Fig.4.6.

i) Pre-Metamorphic Phase

Pre-metamorphic phase includes the tadpoles of limbless stages (stages 22-26).

Stage 22 (Fig.4.5A) "Tail fin circulation stage"

Newly hatched tadpoles are about 5.75 mm (5.0-6.5 mm) in length and their mean weight is 0.028 g (0.020 g - 0.036 g). At the time of hatching they have well developed elongated finger shaped gills and a rudimentary mouth. The gut and anal openings are clearly visible and the body is divisible into head, trunk and tail regions. The tadpoles do not feed at this stage. The tail length/body length ratio at this stage is 1.09. (3.0/2.75).

Stage 23(Fig.4.5B) "Opercular Fold Stage"

The tadpoles possess characters similar to that of stage 22 except that the average body length increases to 7.0 mm (6.0 mm - 7.5 mm) and mean weight to 0.040 g (0.030 g - 0.054 g). The opercular fold becomes wide and the oral papillae become very distinct, but the tadpoles do not feed at this stage also. The tail length/body length ratio at this stage is 1.33 (4.0/3.0).

Stage 24(Fig.4.5C) "Opercular fold closed on the right side stage"

At this stage the average body length increases to 8.0mm (7.0 mm - 9.0 mm) and mean weight to 0.050 g (0.040 g - 0.064 g). The opercular fold grows and completely covers the gills on the right hand side where as the gills are seen partly on the left hand side. The mouth becomes wider and shows two rows of minute horny teeth, but no feeding activity is seen. The tail length/body length ratio at this stage is 1.46 (4.75/3.25).

Stage 25(Fig.4.5D) "Operculum closed and complete stage"

The average body length at this stage is 11.0 mm (8.0 mm - 13.0 mm) and the mean weight is 0.060 g. (0.050 g - 0.076g). The operculum at this stage is seen completely covering the gills on both the sides. The distinction between the head and trunk is no longer visible and the eyes at this stage are prominent. Melanophores can be seen at this stage and are present in characteristic pattern. The tadpoles start feeding at this mainly on detritus plant materials and small organisms such as Diatoms and Rotifers. The tail length/body length ratio is 1.75 (7.0 / 4.0).

Stage 26(Fig.4.5E) "Hind Limb Bud Stage"

At this stage the average body length of the tadpoles increases to 16.5 mm (12.0 mm - 20.0 mm) and mean weight is 0.085 g (0.064 g - 0.116 g). Hind limb appears as a small whitish bud like structure at the junction of the trunk and tail regions. The lateral line system is present but not well developed. Distinct pigmentation pattern is present. The tadpoles feed actively on detritus, plant materials and larger food items such as Algae, Ostrocods, and Zooplanktons. The tail length/body length ratio is 1.75(10.5/6.0).

ii) Pro-Metamorphic Phase

Pro-metamorphic stage is characterised by growth and differentiation of the hind limbs and includes stage 27.

Stage 27(Fig.4.5F) "Hind Limbs Developed Stage"

The average body length of the tadpoles at this stage is 26.0 mm. (20.0 mm - 32.0 mm) and mean weight is 0.170 g (0.116 g - 0.214 g). By the end of this phase, the hind limbs are well developed with distinct thigh, ankle and shaft regions and are used for locomotion. The toes have 5 digits. The tadpoles are seen feeding voraciously at this stage. The tail length/body length is 1.74 (16.5/9.5).

iii) Metamorphic Climax Phase

The metamorphic climax phase includes stages 28 to 31. They are characterised by growth and differentiation of the fore limbs.

Stage 28 (Fig.4.5G) "Fore limb bud stage"

The average body length at this stage is 33.0 mm (30.0 mm - 36.0 mm) and mean weight is 0.224 g (0.194 g - 0.260 g). The fore limbs appear as buds from under the operculum on the lateral sides, whereas the hind limbs are well developed and are used for locomotion. The tadpoles feed at this stage also. The tail length/body length ratio is 1.75 (21.0/12.0).

Stage 29 (Fig.4.5H) "Fore limbs developed stage"

The average body length at this stage reduces to 32.5 mm (34.0 mm - 31.0 mm) and the mean weight is 0.230g (0.260 g - 0.198 g). The fore limb buds become well differentiated, but the tail becomes darker and is also reduced in size. The horny teeth of the mouth are shed and the mouth now appears somewhat like that of the adult stage. The tadpoles continue to feed at this stage also. The tail length/body length ratio is 1.71 (20.5/12.0).

Stage 30 (Fig.4.5I) "Tail shortened stage"

At this stage the average body length is reduced to 29.0 mm (32.0 mm - 26.0 mm) and mean weight to 0.220 g (0.252 g - 0.195 g). The tail is shortened and the angle of the mouth reaches the posterior of the eye ball. The tadpoles stop feeding at this stage. The tail length/body length ratio is 1.52 (17.5/11.5).

Stage 31 (Fig. 4.5J)

At this stage the average body length is reduced to

TABLE - 4.2 MORPHOMETRIC MEASUREMENTS DURING DEVELOPMENTAL STAGES OF RANA LIMNOCHARIS*

Classified Stages	Developmental Stage	Time From Hatching (Days)	Duration of classified stages (days)	Body Weight (mm)		Total Length (mm)		Body Length (mm)		Tail length (mm)		Tail length / Body length ratio
				Range	Mean	Range	Average	Range	Average	Range	Average	
Pre-metamorphic phase	22	2		0.020-0.040	0.028	5.0- 6.5	5.75	2.5-3.0	2.75	2.5- 3.5	3.0	1.09
	23	5		0.030-0.054	0.040	6.0- 7.5	7.0	3.0-3.0	3.0	3.0- 4.5	4.0	1.33
	24	6	7	0.040-0.064	0.050	7.0- 9.0	8.0	3.0-3.5	3.25	4.0- 5.5	4.75	1.46
	25	7		0.050-0.076	0.060	8.0-13.0	11.0	3.5-4.5	4.0	4.5- 8.5	7.0	1.75
	26	9		0.064-0.116	0.085	12.0-20.0	16.5	4.5-7.5	6.0	7.5-12.5	10.5	1.75
Pre-metamorphic phase	27	13	7	0.116-0.214	0.170	20.0-32.0	26.0	7.5-11.0	9.5	13.5-21.0	16.5	1.74
Metamorphic Climax	28	20		0.194-0.260	0.224	30.0-36.0	33.0	11.0-13.0	12.0	19.0-23.0	21.0	1.75
	29	25		0.260-0.191	0.230	34.0-31.0	32.5	13.0-11.0	12.0	21.0-20.0	20.5	1.71
	30	27	16	0.252-0.195	0.220	32.0-26.0	29.0	12.0-11.0	11.5	20.0-15.0	17.	1.52
	32			0.220-0.180	0.202	27.0-13.0	19.0	11.5-11.0	11.25	15.5-4.0	7.75	0.69
Metamorphosed Juvenile	32	32	3	0.210-0.140	0.170	14.0-9.0	11.0	-	-	-	-	-

*Stages based on Roy and Khare, 1978.

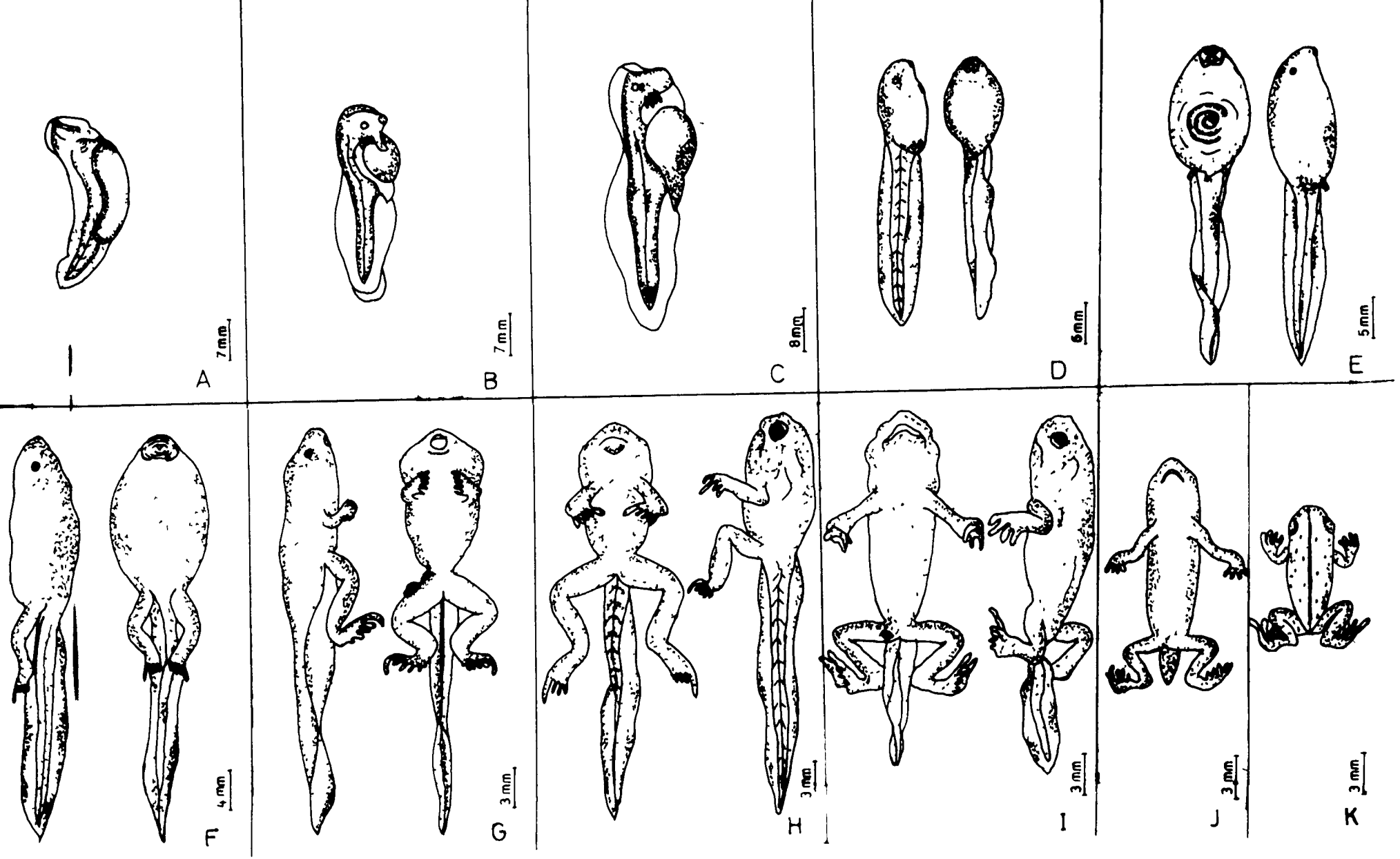


FIG 4 5 NORMAL DEVELOPMENTAL STAGES OF *Rana limnocharis*

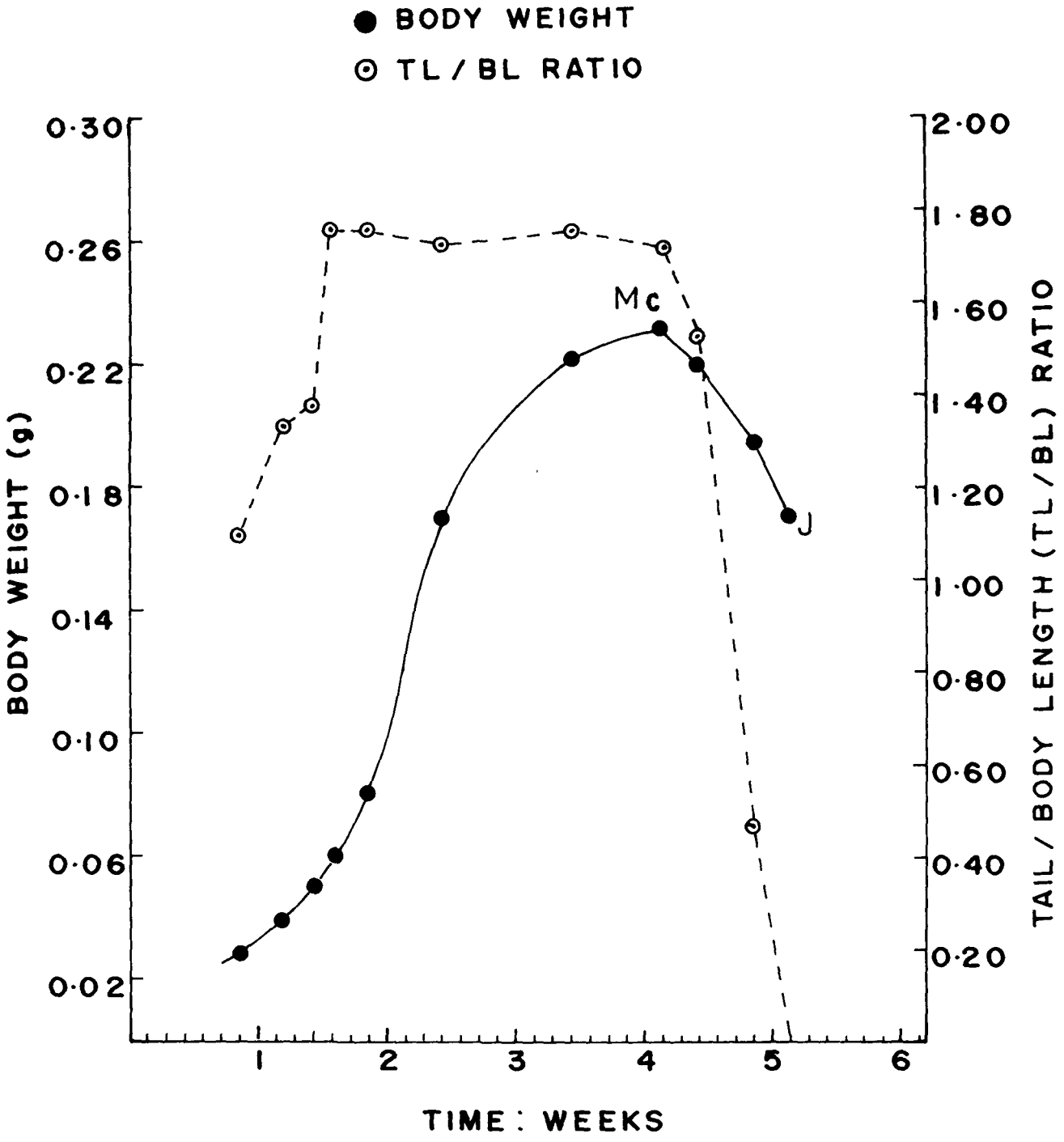


FIG. 4.6 GROWTH AND DIFFERENTATION OF *Rana limnocharis* TADPOLES (Mc = METAMORPHIC CLIMAX STAGE, J = METAMORPHOSED JUVENILE)

19.0 mm. (27.0 mm - 13.0 mm) and mean weight to 0.202 g (0.220 g - 0.180 g). The tail is present only as a small stump. Several drastic metamorphic transformations begin and the tadpoles now foreshadow the adult characteristics and more or less resemble them, excepting the size. The tadpoles do not feed at this stage. The tail length/body length ratio is reduced to 0.69 (7.75/11.25).

Metamorphosed Juveniles

Stage 32 (Fig.4.5K)

At this stage several drastic transformations which began at stage 31 are completed and the froglets attain characteristics for amphibious or semiterrestrial life, like that of the adults. The average snout-vent length of the froglets is 11.0 mm. (14.0 mm - 9.0 mm) and the tail is completely resorbed by this stage.

4.2.5 Description of Tadpoles

The characteristic features of the tadpoles of *Rana limnocharis* are well developed at stage 27 of Roy and Khare (1978). This stage is comparable to Gosners (1960) stage 38. As per the criteria of Van Dijk (1966) the tadpoles of this stage are used for identification of species.

a) Morphometric Measurements

Various morphometric measurements of tadpoles at stage 27 have been compiled in Table 4.3. The total body length ranged from 20.0 to 32.0 mm ($\bar{x}$ 26.0 mm) while the body length and tail

TABLE 4.3

Measurements of tadpoles of *Rana limnocharis* at stage 27.

BODY PARTS	RANGE	MEAN
Total body length - mm	20.0-32.0	26.0
Body length - mm	7.5-11.0	9.5
Tail length - mm	12.5-21.0	16.5
Body weight - g	0.116-0.214	0.170
Gut weight - g	0.045-0.075	0.060
Gut content weight - g	0.030-0.060	0.045

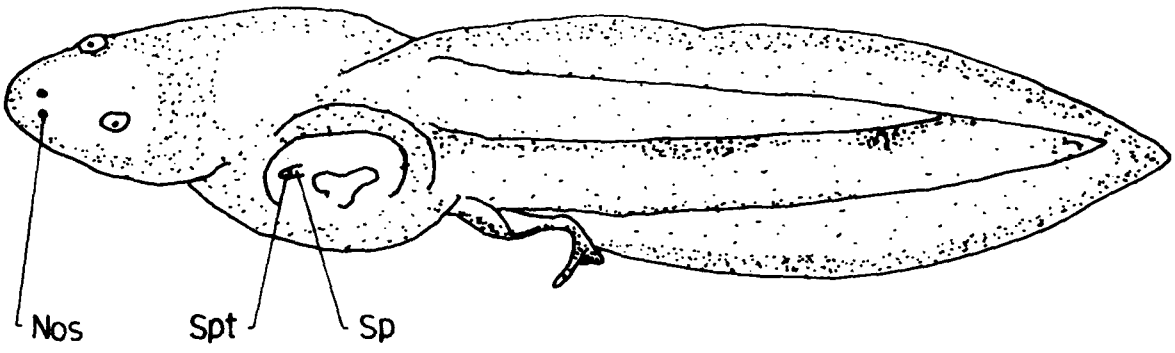
Sample size, n = 30

length ranged from 7.5 to 11.0 mm ($\bar{x}$ 9.5 mm) and 12.5 to 21.0 mm ($\bar{x}$ 16.5 mm) respectively. The weight of the gut ranged from 0.045g to 0.075g ($\bar{x}$ 0.060 g)and weight or gut contents ranged from 0.030 g to 0.060 g ($\bar{x}$ 0.045 g).

b) Morphological characteristics

Various morphological characteristics of tadpoles of *Rana limnocharis* tadpoles based on an analysis of a sample of 30 tadpoles are given below (see Fig.4.7).

Body Size (Total body length)	:	Body size ranges from 20.0 mm to 32.0 mm). ($\bar{x}$ 26.0 mm).
Body shape	:	More or less ovoid with pointed snout.
Mouth	:	Subterminal with rounded opening.
Oral disc	:	Emarginate in nature. Width ranges from 1.7 mm to 1.9 mm. Single row of marginal oral papillae Rostral (1.9 mm) and mental (1.0 mm) gaps present. Supra and infra angular intra marginal oral papillae arranged in two rows. Supra rostrodont with lateral inflexions and serrated along entire margins. Serrations are blunt and keratinized. (0.02 mm long and 0.016 mm wide).
Dental formula	:	1 + 1/3.



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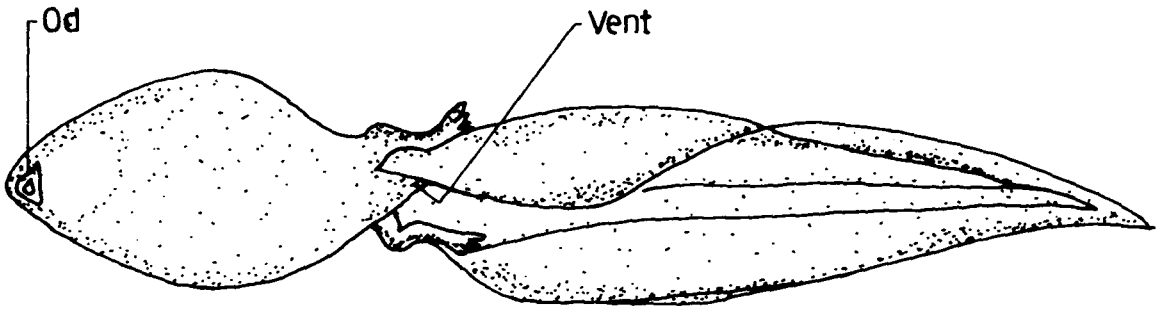

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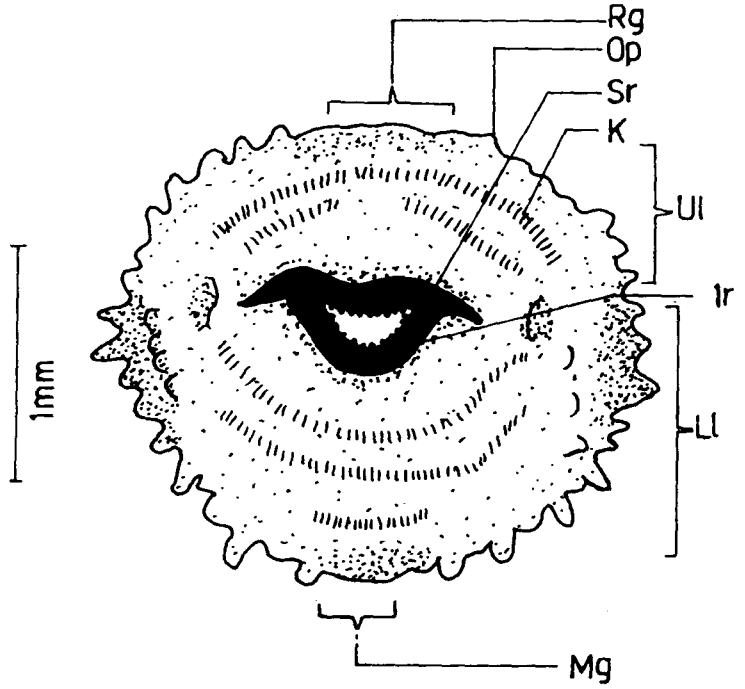

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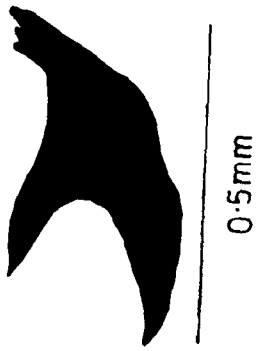

1D

FIG. 4.7 IDENTIFYING CHARACTERISTICS OF *Rana limnocharis*:st.27. TADPOLES. (AFTER SAHU, 1981). A = LATERAL VIEW, B=VENTRAL VIEW, C= ORAL DISC, D = SINGLE KERATODONT Nos = NOSTRILS, Spt. = SPIRACULAR TUBE, Sp=SPIRACLE, Rg = ROSTRAL GAP, Op = ORAL PAPILLAE, Sr= SUPRA ROSTRODONT K = KERATODONT, Ul = UPPER LABIUM, Ir = INFRA ROSTRODONT, Ll = LOWER LABIUM, Mg = MENTAL GAP.

Tail	:	Tip is pointed Height of the tail equals height of trunk. Tail axis extrapolated forward to the middle of the eyes. Tail axis is thin yellowish and curves upwards slightly behind the middle of the tail.
Pigmentation	:	Melanophores numerous on dorsal side. Ventral side light dark with superficial melanophores. Tail fins with scattered groups of melanophores. Pigmentation around nostrils is heavy.
Eyes	:	Dorsolateral in position.
Vent	:	Caudal and dextral in position.

c) Morphometric Ratios

Various morphometric ratios useful for identification of tadpoles have been presented in Table 4.4.

4.3 EXPERIMENTS IN RELATION TO FOOD

4.3.1 Analysis of gut contents of fully developed tadpoles At Stage 27.

The gut contents of the tadpoles have been examined at stage 27. An analysis of the periphyton samples of their habitat has also been carried out for comparison.

TABLE 4.4

VARIOUS MORPHOMETRIC RATIOS OF THE TADPOLES OF *Rana Limnocharis* AT STAGE 27

1. Distance between nostrils	*Internarial distance / Nostril Width*	*3.9*
2. Extranarial proportion	*Width of the head minus distance between lateral margins of nostrils / Distance between lateral margins of nostrils*	*1.6-1.9($\bar{x}$ 1.75)*
3. Longitudinal position of the nostrils in relation to the eyes	*Rostronasal distance / Orbitonasal distance*	*1.0-1.9($\bar{x}$ 1.4)*
4. Extraocular proportion	*Width of the head minus distance between lateral margins of the eyes / Distance between the lateral margins of the eyes*	*0.32-0.41($\bar{x}$ 0.37)*
5. Spiracular position anteroposteriorly	*Distance from rostrum to the centre of the spiracular opening / Distance from the rostrum to posterior limit of the trunk*	*0.44-0.62($\bar{x}$ 0.53)*
a. Posterior displacement along the trunk *b. Posterior displacement along the tail*	*Distance from posterior limits of trunk to the centre of spiracular opening / Distance from centre of spiracular opening to the tip of the tail*	*0.22-0.30($\bar{x}$ 0.26)*
6. Vent Ratio	*Proctodael tube length / Vent width*	*2.0*
7. Tail length	*Tail length / Length of head and trunk*	*1.3-1.7($\bar{x}$ 1.5)*
8. Tail height	*Tail height / Height of the trunk*	*1.0*

Sample size, n = 30

A) *Gut contents*

The contents of the fore gut and hind gut were analysed seperately in each of the cases and expressed as percentage per 0.1 ml of the sample. The food items were identified by works of Edmenson (1959).

a) Percentage of major groups of food items

The percentage of the major food items examined have been compiled in Table 4.5.

Fore gut

Chlorophyceae constituted 60.8% which was the highest among various food items. Bacillariophyceae constituted 18.2% and those of Cyanophyceae and Non algal forms were 6.4% and 14.6% respectively. Detritus, Vascular plant materials and mud were present in abundant quantities.

Hind gut

In the hind gut Chlorophyceae constituted 45.3% which was also the highest among various food items, while Bacillariophyceae constituted 16.3%. Cyanophyceae and Non algal forms constituted 24.6% and 13.8% respectively. Detritus, vascular plant materials and mud were present in abundant quantities.

Periphyton sample

Chlorophyceae constituted 42.1% in the periphyton sample, while Bacillariophyceae constituted 35.3%, Cyanophyceae and Non algal forms constituted 5.1% and 17.5% respectively.

TABLE 4.5

TOTAL PERCENTAGE COMPOSITION OF MAJOR GROUPS OF FOOD ITEMS/0.1 ml OF PERIPHYTON, FORE GUT AND HIND GUT SAMPLES OF *Rana limnocharis* TADPOLES AT STAGE 27.

MAJOR FOOD ITEMS	PERIPHYTON-%	FOREGUT-%	HINDGUT-%
Chlorophyceae	42.1	60.8	45.3
Bacillariophyceae	35.3	18.2	16.3
Cyanophyceae	5.1	6.4	24.6
Non-algal forms	17.5	14.6	13.8

Sample size, n = 30

b) *Percentage of Individual Food Items*

The percentage of occurrence of various food items have been compiled in Table 4.6.

Fore gut

The order of occurrence of food items in the fore gut was *Chlorophyceae :* Ankistrodesmus > Oedogonium > Scenedesmus > Closterium>Penium > Spirogyra > Zygnema > Ulothrix,*Bacillariophyceae:* Navicula > Neidium > Opephora > Amphora > Pinnularia,*Cyanophyceae:* Oscillatoria > Anabena and *Non algal forms :*Rhizopoda > Bacteria> Ostrocoda > Protoza > Daphnia

Hind gut

The order of occurrence of food items in the fore gut was *Chlorophyceae :* Ankistrodesmus > Oedogonium > Scenedesmus > Spirogyra > Closterium > Penium > Ulothrix > Zygnema, *Bacillariophyceae :* Navicula > Neidium > Opephora > Amphora > Pinnularia, *Cyanophyceae :*Oscillatoria > Anabena and *Non algal forms :* Rhizopoda>Protoza > Bacteria > Rotifera > Ostrocoda> Bosmina.

Periphyton

The order of occurrence of food items in the periphyton samples *Chlorophyceae :* Ankistrodesmus > Scenedesmus > Oedogonium > Ulothrix > Closterium > Zygnema > spirogyra > Penium; *Bacillariophyceae :* Navicula > Opephora > Amphora > Neidium > Pinnularia; *Cyanophyceae :* Ocillatroia > Anabena and *Non algal forms :* Bacteria > Cladocera > Protozoa.

TABLE : 4.6

PERCENTAGE COMPOSITION OF INDIVIDUAL FOOD ITEMS PER 0.1 mL OF THE GUT CONTENTS OF TADPOLES OF *Rana limnocharis* AT STAGE 27, AND PERIPHYTON SAMPLE

FOOD ITEMS	FORE GUT	HIND GUT	PERIPHYTON
Chlorophyceae			
Ankistrodesmus	17.3	17.6	14.2
Spirogyra	3.8	4.1	2.3
Ulothrix	3.2	2.2	4.5
Zygnema	3.5	1.6	2.3
Oedogonium	10.6	7.3	5.9
Closterium	6.8	3.8	2.5
Scenedesmus	9.1	6.2	8.6
Penium	6.5	2.5	1.8
Bacillariophyceae			
Navicula	9.4	10.4	22.8
Amphora	1.8	1.2	3.4
Pinnularia	1.2	0.8	1.8
Opephora	2.6	1.5	4.2
Neidium	3.2	2.4	3.1
Cyanophyceae			
Anabaena	1.2	2.4	0.6
Oscillatoria	5.2	22.2	4.5
Non algal forms			
Bacteria	3.5	2.4	2.8
Protoza	0.8	4.5	0.5
Rotifera	1.0	0.8	--
Cladocera	0.5	0.6	1.0
Ostracoda	1.2	0.7	--
Rhizopoda	7.6	4.8	13.2

Sample size, n - 30

The analysis of gut contents shows that in both the fore gut and hind gut Chlorophyceae constituted the highest percentage among different groups of food items. This was followed by Bacillariophyceae, Non algal forms and Cyanophyceae. The same order of occurrance was observed in the periphyton sample.

The total percentage of phytoplanktons in the fore gut was 92.3% and in the hind gut it was 91.8%, while zooplanktons in the fore gut were 7.7% and in the hind gut 8.2%.

The presence of large amounts of detritus, vascular plant materials and mud in the gut contents indicates that the tadpoles primarly feed on the lower or bottom regions of the ponds and pools. Also the observations of gut contents, of the hind gut indicates that most of the food items were intact but without their cell contents; suggesting that the tadpoles consume only the cell contents of the food items.

*4.3.2 *Analysis of gut contents at different developmental stages: in relation to development of mouth parts*

The gut contents of tadpoles of stages ranging from 21 to 32 have been examined and development of mouth parts and relationship of types of food taken at these stages has been investigated.

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* Paper presented at the (4th Ordinary General Meeting) International Session of the "Societas Europaea Herpetologica", Nijmegen, Netherland, 1987.

For the sake of convenience tadpoles were grouped into 3 broad categories namely (1) Pre-feeding stages (2) Active feeding stages and (3) Post-feeding stages. The changes in mouth parts and food items consumed at different developmental stages has been complied in Table 4.7.

Pre-Feeding Stages (Stages 21- 24)

a) Gut contents

During the prefeeding stages no feeding activity was seen and the tadpoles derieve energy from the stored food materials. The gut during these stages contains yolk material.

b) Mouth parts

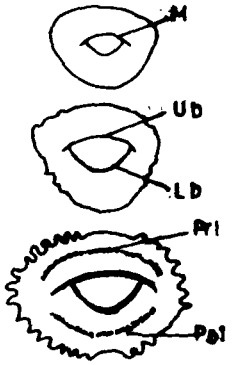
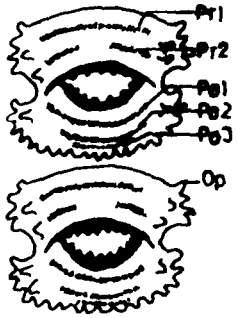
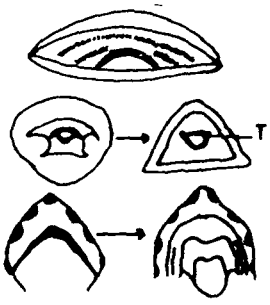
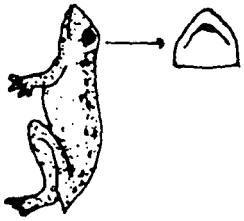
The development of mouth parts begins as a small triangular slit (0.2-0.4 mm) situated on the anterio ventral side of the tadpoles.

At stage 21 the mouth is small (0.4 mm - 0.5 mm) and triangular in shape.

At stages 22-23, the mouth becomes slightly wide (0.5-1.0 mm) and the upper and lower labial jaws appear with faintly serrated margins. The upper and lower labial fringes develop some oral papillae.

At stage 24, the mouth becomes more or less oval in shape (1.00 - 2.00 mm). The horny beaks become dark black in colour and exhibit distinct serrations. In addition to this two rows of minute horny teeth are seen at this stage and the dental formula is 1/1.

TABLE 4.7 FOOD ITEMS, DENTAL FORMULA AND ORAL STRUCTURES AT DIFFERENT DEVELOPMENTAL STAGES OF *Rana limnocharis*

Category	Stages	Food items	Dental formula	Oral structures
Pre-feeding stages	21	Feed on stored food materials (yolk)	$\frac{0}{0}$	
	22-23	- ,, -	Upper and lower beaks with faintly serrated margins	
	24	-,,-	$\frac{1}{1}$	
Active feeding stages	25	Detritus, Mud, Vascular plant material Rotifers Diatoms	$\frac{1}{1+1}{3}$	
	26-28	Detritus Mud, Vascular plant materials, Ostracods Filamentous algae	$\frac{1}{1+1}{3}$	
Post feeding stages	29	Detritus, Mud Vascular plant materials, Filamentous algae, Ostracods	$\frac{1}{1+1}{3}$	
	30-31	No feeding activity is seen during these stages	----	
Metamorphosed froglets		Formicoidea Oligochaeta, Coleoptra Insect larvae, Arachnids.	----	

Active feeding stages (Stages 25-28)

a) Gut contents

The tadpoles start feeding from stage 25. They are now seen actively consuming food materials. An analysis of gut contents at this stage indicates the presence of detritus, vascular plant materials and some Non algal forms such as diatoms and Rotifers.

The gut contents of the tadpoles at stages 26-28 mainly include large amounts of detritus, sand and mud, vascular plant materials, Ostracods, Zooplanktons and some large food items such as filamentous algae.

b) Mouth Parts

The tadpoles start feeding at stage 25. At this stage they pocess a complete set of teeth rows. The mouth is wide and elongated in shape (2.0 - 3.0mm). There is one continuous row and one interrupted row of teeth on the upper labial fringe,. while the lower labial fringe has 3 faint but distinct rows of teeth.

At stages 26-28, the mouth is wide (3.0 - 4.0 mm) and has very well developed mouth parts with a dental formula of 1:1+1/3.

Post feeding stages (Stages 29-32)

a) Gut contents

The tadpoles usually stop feeding or consume very little

food which mainly consists of detritus, sand and mud, vascular plant materials and non algal forms (Zooplanktons).

At the metamorphic climax stages (stages 30-31), they do not feed at all. An analysis of gut indicates that it is mostly empty and contains detritus and some digested food materials.

b) Mouth parts

At stage 29, the labial fringes bearing teeth rows are clearly seen but a few teeth appear to have been shed and the mouth appears modified somewhat like that of adult.

At stage 30 all teeth are shed and by stage 31 metamorphosis is almost complete and the mouth now resembles the adult type with a prominent tongue.

At stage 32 metamorphosis is complete and the juveniles start living an amphibious life. The mouth is well developed with teeth and a prominent tongue.

Analysis of gut contents indicates that Formicoides formed the highest percentage among various food items. This was followed by Oligochaetes, Coleopterans, Insect larvae and Arachnids.

The observations reveal that at early pre-feeding stages (stages 22-24) no feeding activity is seen and the mouth parts are not well developed. At stage 25, the tadpoles start feeding

on detritus, vascular plant materials and some non algal forms such as Diatoms and Rotifers. At stages 26-28, the tadpoles are seen feeding actively on detritus, vascular plant materials and some large food items such as filamentous algae, Ostrocods and Zooplanktons. At stage 29 very little feeding activity is seen and at stages 30-31 there is no feeding activity seen at all. Various mouth parts are either shed or modified to suit a carnivorous habit. Thus a relationship between the size of food items consumed and size and shape of the mouth exists.

4.3.3 Analysis of growth with artificial diet

i) Quality of food

The present experiment was conducted to see the effect of six different types of artificial food items namely boiled cabbage, boiled lettuce, boiled egg yolk, cooked goat meat, ripe banana and mixed food (combination of all food items) on the growth and metamorphosis of the tadpoles hatched in the laboratory.

The tadpoles of the same mean age, size and weight (2 d old, weight 0.025 g, average length 5.75 mm) were randomly separated into groups of 15 tadpoles each and each group was given a different food. The growth values of the tadpoles recorded twice a week have been plotted in a graph (Fig.4.8).

Boiled cabbage and boiled lettuce

It was observed that tadpoles fed with boiled cabbage and boiled lettuce attained a mean weight of 0.230 g (0.198-0.260g) and

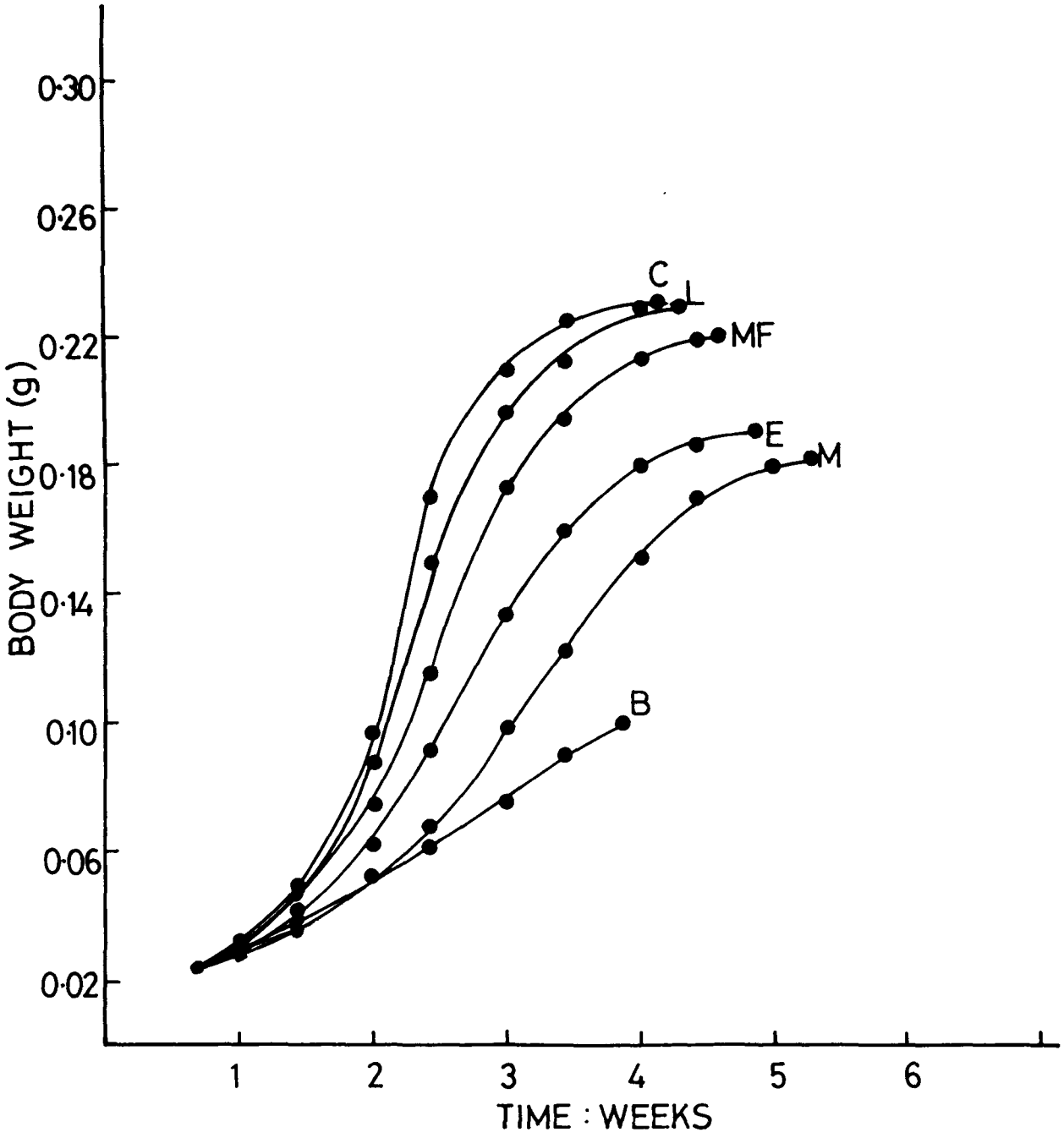


FIG. 4.8 EFFECT OF FOOD QUALITY ON GROWTH OF *Rana limnocharis* TADPOLES.
(C=CABBAGE, L = LETTUCE, MF = MIXED FOOD, E = BOILED EGG YOLK, M = COOKED GOAT MEAT, B = BANANA.)

0.228 g (2.000 g - 0.252g) respectively, on 29 to 30 days of growth and metamorphosed by 36 to 38 days.

Mixed food

The tadpoles fed with mixed food (combination of all food items) attained a mean weight of 0.220 g (0.200 g - 0.248 g) on 32 days of growth and metamorphosed by 39 to 40 days.

Boiled egg yolk and cooked goat meat

The tadpoles fed with boiled egg yolk and cooked goat meat attained a mean weight of 0.190 g (0.174 g - 0.212 g) and 0.182 g (0.150 g - 0.206 g) respectively on 34 to 37 days and metamorphosed by 41 to 44 days.

Ripe Banana

All the tadpoles fed with ripe banana died by the 27th day of growth, at which time their mean weight was 0.100 g (0.888 - 0.124 g).

The experimental data shows that tadpoles fed with either boiled cabbage or boiled lettuce grow faster and metamorphose earlier (36-38 days), than the tadpoles fed on other food items such as boiled egg yolk , cooked goat meat, ripe banana and mixed food (39-56 days).

Analysis of variance

A one way analysis of variance has been applied to determine

the effect of different artificial diets on the growth and metamorphosis of tadpoles.

Growth

The mean weight of the tadpoles in each of the treatments was calculated on the 24th day of growth, the time when maximum growth had been attained. The mean weight, range and SD of the tadpoles are compiled in Table 4.8. The analysis of variance (Table 4.9) shows that for 5 degrees of freedom between groups (greater variance) and 12 degrees of freedom within groups (smaller variance), the critical value of F at 0.01 level of significance is 4.46. The calculated value of F in the present analysis is 10.89 which is more than it. Hence there is a significant difference in growth rate of tadpoles when fed on different diets.

Metamorphosis

The mean weight, range and SD of the tadpoles at the metamorphic climax are compiled in Table 4.10. The analysis of variance (Table 4.11) indicates that the calculated value of F (11.17) in the present analysis is greater than the critical value of F at 0.01 level of significance and hence different food items significantly affect the body weight at metamorphic climax.

The curves (Fig. 4.8) statistical analysis of growth rate (Table 4.9) and body weight at metamorphic climax (Table 4.11)

TABLE - 4.8 MEAN BODY WEIGHT OF *Rana limnocharis* TADPOLES AFTER 24 DAYS OF GROWTH AS A FUNCTION OF DIFFERENT DIETS.

DIET	RANGE (g)	MEAN (g)	± SD
Cabbage	0.194-0.260	0.225	0.033
Lettuce	0.180-0.242	0.212	0.029
Egg	0.130-0.178	0.160	0.026
Meat	0.094-0.146	0.122	0.026
Banana	0.067-0.108	0.090	0.020
Mixed food	0.164-0.220	0.194	0.028

Mean weight of tadpoles at stage 22=0.025 g.

TABLE 4.9 ONE WAY ANALYSIS OF VARIANCE OF MEAN BODY WEIGHT(G) OF *Rana limnocharis* TADPOLES AFTER 24 DAYS OF GROWTH AS A FUNCTION OF DIFFERENT DIETS

	FOOD ITEMS (a = 6)					
REPLICATES	CABBAGE	LETTUCE	EGG	MEAT	BANANA	MIXED FOOD
1	0.194	0.180	0.172	0.094	0.095	0.220
2	0.260	0.242	0.178	0.146	0.067	0.164
3	0.221	0.214	0.130	0.126	0.108	0.198

COMPUTATIONS

a) Grand Total, T = 3.009
b) Correction factor, T^2/N = 0.503004
c) Total sum of squares of all items = 0.554711
d) Total sum of squares (SST) = 0.51707
e) Sum of squares between groups (SSC) = 0.042363
f) Sum of squares within groups (SSE) = 0.009344

CALCULATION OF F-RATIO - ANOVA TABLE

SOURCE OF VARIATION	SUM OF SQUARES	DEGREE OF FREEDOM	MEAN SQUARES	F-RATIO
BETWEEN GROUPS (treatments)	0.042363	5	0.008473	10.89*
WITHIN GROUPS (error)	0.009344	12	0.000778	
TOTAL	0.075289	17		

* F Significant at 0.01 level.

TABLE - 4.10 MEAN BODY WEIGHT OF *Rana limnocharis* TADPOLES AT METAMORPHIC CLIMAX AS A FUNCTION OF DIFFERENT DIETS:

DIET	RANGE (g)	MEAN (g)	± SD
Cabbage	0.198-0.260	0.230	0.031
Lettuce	0.200-0.252	0.228	0.026
Egg	0.174-0.212	0.190	0.020
Meat	0.150-0.206	0.182	0.028
Banana	0.088-0.124	0.100	0.020
Mixed food	0.200-0.248	0.220	0.025

TABLE 4.11 ONE WAY ANALYSIS OF VARIANCE OF MEAN BODY WEIGHT(G) OF *Rana limnocharis* TADPOLES AT METAMORPHIC CLIMAX A FUNCTION OF DIFFERENT DIETS

	FOOD ITEMS (a = 6)					
REPLICATES	CABBAGE	LETTUCE	EGG	MEAT	BANANA	MIXED FOOD
1	0.198	0.252	0.172	0.152	0.088	0.248
2	0.260	0.200	0.186	0.190	0.090	0.200
3	0.234	0.232	0.212	0.206	0.124	0.212

COMPUTATIONS

a) Grand Total, T = 3.456
b) Correction factor, T^2/N = 0.663552
c) Total sum of squares of all items = 0.70732
d) Total sum of squares (SST) = 0.043768
e) Sum of squares between groups (SSC) = 0.036024
f) Sum of squares within groups (SSE) - 0.007744

CALCULATION OF F-RATIO - ANOVA TABLE

SOURCE OF VARIATION	SUM OF SQUARES	DEGREE OF FREEDOM	MEAN SQUARES	F-RATIO
BETWEEN GROUPS (treatments)	0.036025	5	0.007205	11.17*
WITHIN GROUPS (error)	0.007744	12	0.000645	
TOTAL	0.043768	17		

*F significant at 0.01 level.

indicates that different artificial food items influence the growth rate of tadpoles and hence reflected at metamorphic climax. In the present experiment boiled cabbage and boiled lettuce were found to be suitable for feeding of tadpoles in laboratory. Apparently no deformities were seen in the tadpoles. However it was observed that small amounts of proteinaceous food such as boiled egg yolk can be added during the later stages of development.

ii) Quantity of Food

As in the previous experiment the growth of the tadpoles fed on cabbage was best, the present experiment was conducted to see the effect of amount of food (cabbage) consumed on the growth and metamorphosis of tadpoles of *Rana limnocharis*.

In the present experiment six different food quantities 0.1 g , 0.2 g, 0.4 g, 0.6 g, 0.8 g and 1.0 g were given for each set of 5 tadpoles kept in 1.0 l of the medium and changed every 2 days. The growth rate has been illustrated in Fig.4.9. Visual inspection of the growth curves indicates that the tadpoles reared with 0.6 g of food showed the highest growth rate. They attained a mean weight of 0.230 g (0.198 g - 0.260 g) and metamorphosed by 36-37 days.

The tadpoles reared with rations of 0.8 g and 1.0 g, attained a mean weight of 0.228 g (0.194 - 0.258 g) and 0.220 g (220 g - 0.248 g) respectively and metamorphosed by 37-38 days.

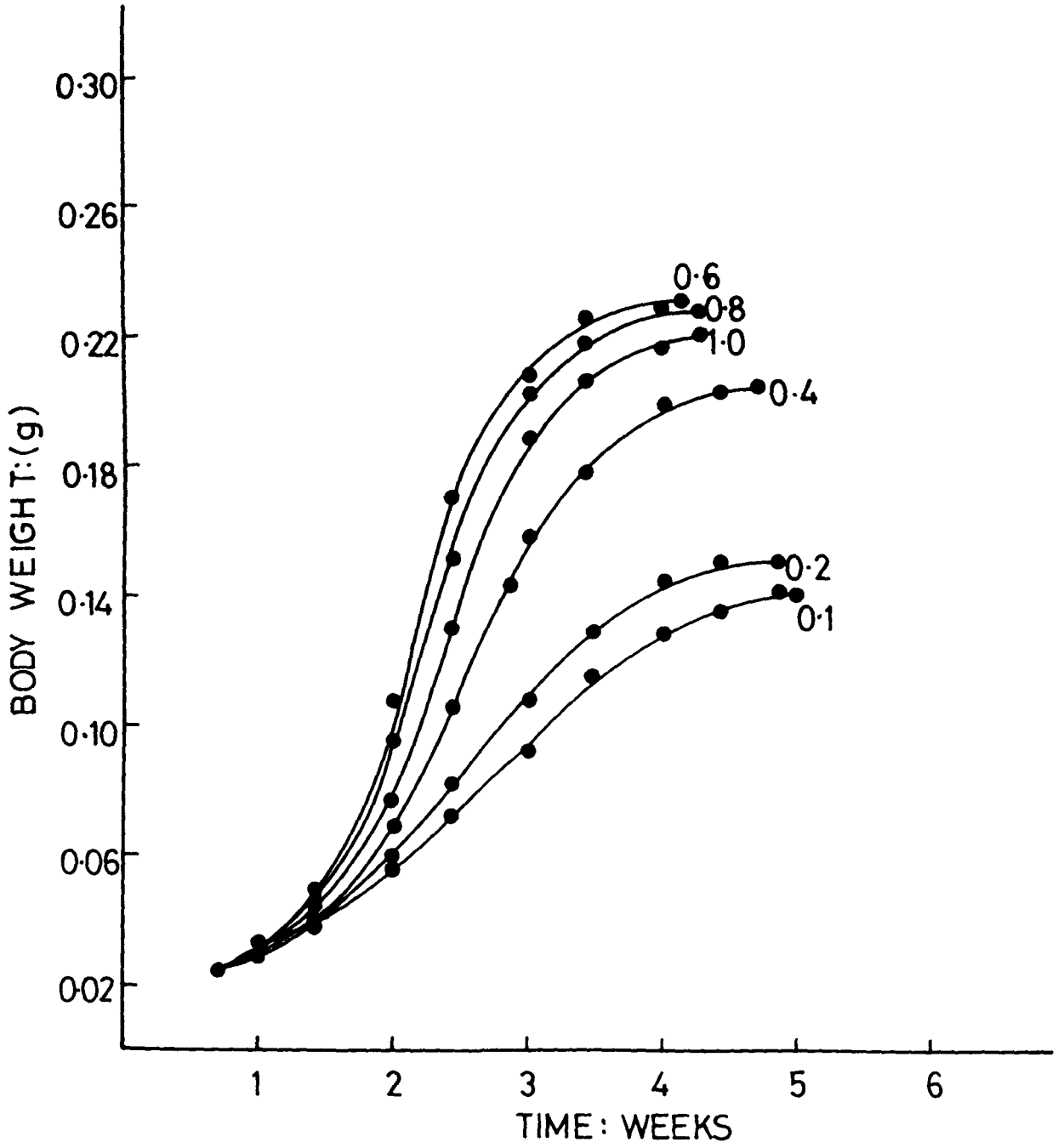


FIG. 4.9. EFFECT OF FOOD QUANTITY ON GROWTH OF *Rana limnocharis* TADPOLES. (NUMBERS INDICATE FOOD LEVELS IN 'g').

The tadpoles reared with a ration of 0.4g attained a mean weight of 0.20 g- (0.175 g- 0.228 g) and metamorphosed by 40-41 days.

Tadpoles reared with a ration of 0.2 and 0.1 g attained a mean weight of 0.150 g (0.134 g- 0.172 g), 0.140 g (0.126 g- 0.158 g) and metamorphosed by 41-42 days.

Analysis of variance

A one way analysis of variance has been applied to determine the effect of different quantities of food on the growth and metamorphosis of tadpoles.

Growth

The weight of the tadpoles in different treatments were calculated on the 24th day of growth, when maximum growth had been attained. The mean weight, range and SD of the tadpoles are compiled in table 4.12. The analysis of variance (Table 4.13) shows that the calculated value of F (11.68) is greater than the critical calue of F at 0.1 level of significance. Hence there is a significant difference in growth rate of tadpoles when different quantities of food are used.

Metamorphosis

The weight of the tadpoles of all treatments were calculated at metamorphic climax stage , and the range, mean and SD are

TABLE - 4.12 MEAN BODY WEIGHT OF *Rana limnocharis* TADPOLES AFTER 24 DAYS OF GROWTH AS A FUNCTION OF FOOD QUANTITIES.

FOOD QUANTITY (g)	BODY WEIGHT (g) RANGE	MEAN	± SD
0.1	0.092 - 0.136	0.114	0.022
0.2	0.102 - 0.146	0.128	0.023
0.4	0.148 - 0.200	0.178	0.027
0.6	0.221 - 0.260	0.194	0.021
0.8	0.196 - 0.254	0.218	0.031
1.0	0.180 - 0.228	0.206	0.024

Mean weight of tadpoles at stage 22 = 0.025g.

TABLE - 4.13 ONE WAY ANALYSIS OF VARIENCE OF MEAN BODY WEIGHT OF *Rana limnocharis* TADPOLES AFTER 24 DAYS OF GROWTH AS A FUNCTION OF FOOD QUANTITY.

REPLICTES	FOOD LEVELS (g)					
	0.1	0.2	0.4	0.6	0.8	1.0
1.	0.0092	0.146	0.186	0.260	0.196	0.210
2.	0.114	0.102	0.148	0.221	0.204	0.228
3	0.136	0.136	0.200	0.225	0.254	0.180

Computations

a) Grand total, T = 3.238
b) Correction factor, T^2/N = 0.582480
c) Total sum of squares of all items = 0.626770
d) Total sum of squares (SST) = 0.44290
e) Sum of squares between groups (SSC) = 0.036737
f) Sum of squares within groups (SSE) = 0.007553

Calculation of F-Ratio - ANOVA table

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F-RATIO
Between groups (treatments)	0.036737	5	0.007347	11.68*
Within groups (error)	0.007553	12	0.000629	
Total	0.044290	17		

*F significant at 0.01 level.

TABLE - 4.14 MEAN BODY WEIGHT OF *Rana limnocharis* TADPOLES AT METAMORPHIC CLIMAX AS A FUNCTION OF FOOD QUANTITY.

FOOD LEVELS (g)	RANGE (g)	MEAN (g)	± SD
0.1	0.126-0.158	0.140	0.016
0.2	0.134-0.172	0.150	0.019
0.4	0.175-0.228	0.204	0.027
0.6	0.198-0.260	0.130	0.031
0.8	0.194-0.258	0.228	0.032
1.0	0.200-0.248	0.220	0.025

TABLE - 4.15 ONE WAY ANALYSIS OF VARIANCE OF MEAN BODY WEIGHT (g) *Rana limnocharis* TADPOLES AT METAMORPHIC CLIMAX AS A FUNCTION OF FOOD QUANTITY.

REPLICATES	FOOD LEVELS (g)					
	0.1	0.2	0.4	0.6	0.8	1.0
1	0.158	0.134	0.175	0.198	0.258	0.200
2.	0.126	0.144	0.228	0.234	0.232	0.248
3.	0.136	0.172	0.209	0.260	0.194	0.212

Computations

a) Grand total, T = 3.518
b) Correction factor, T^2/N = 0.687573
c) Total sum of squares of all items = 0.719934
d) Total sum of squares (SST) = 0.03236
e) Sum of squares between groups (SSC) = 0.24348
f) Sum of squares within groups (SSE) = 0.008013

Calculation of F-Ratio - ANOVA table

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F-Ratio
Between groups (treatments)	0.024348	5	0.004869	7.30*
Within groups (error)	0.008013	12	0.000667	
Total	0.032361	17		

F* significant at 0.01 level

compiled in Table 4.14. The analysis of variance (Table 4.15) indicates that the calculated value of F (7.30) is greater than the critical value of F at 0.01 level of significance and hence the weight of the tadpoles is significantly affected at metamorphic climax as far as food quantity is concernt.

The curves (Fig. 4.8), statistical analysis of growth rate (Table 4.13) and body weight at metamorphic climax (table 4.15)indicates that food quantity influencesthe growth rate and hence reflected at metamorphic climax. In the present experiment 0.6 g level of food was found to be optimum.

4.4 DENSITY RELATED EXPERIMENTS

4.4.1 Tadpoles reared in Isolation

For this experiment tadpoles were hatched in the laboratory and on the second day of hatching (2 d old, av., length, 5.75 mm, mean weight 0.025 g) were seperated and reared individually. The mean weight of the growing tadpoles were recorded twice a week on the basis of 20 replicates. The data has been plotted in a graph (Fig. 4.10). Initially at the start of the experiment the mean weight of the tadpoles was 0.025 g. It increased gradually during the first week of pre-metamorphic phase to 0.040 g and then sharply from 10 to 21 days to 0.180 g. During the pro-metamorphic phase the increasein mean weight slowed down so that at metamorphic climax the maximum mean weight recorded was 0.220 g and was attained by 28 to 30 days. This was followed by a

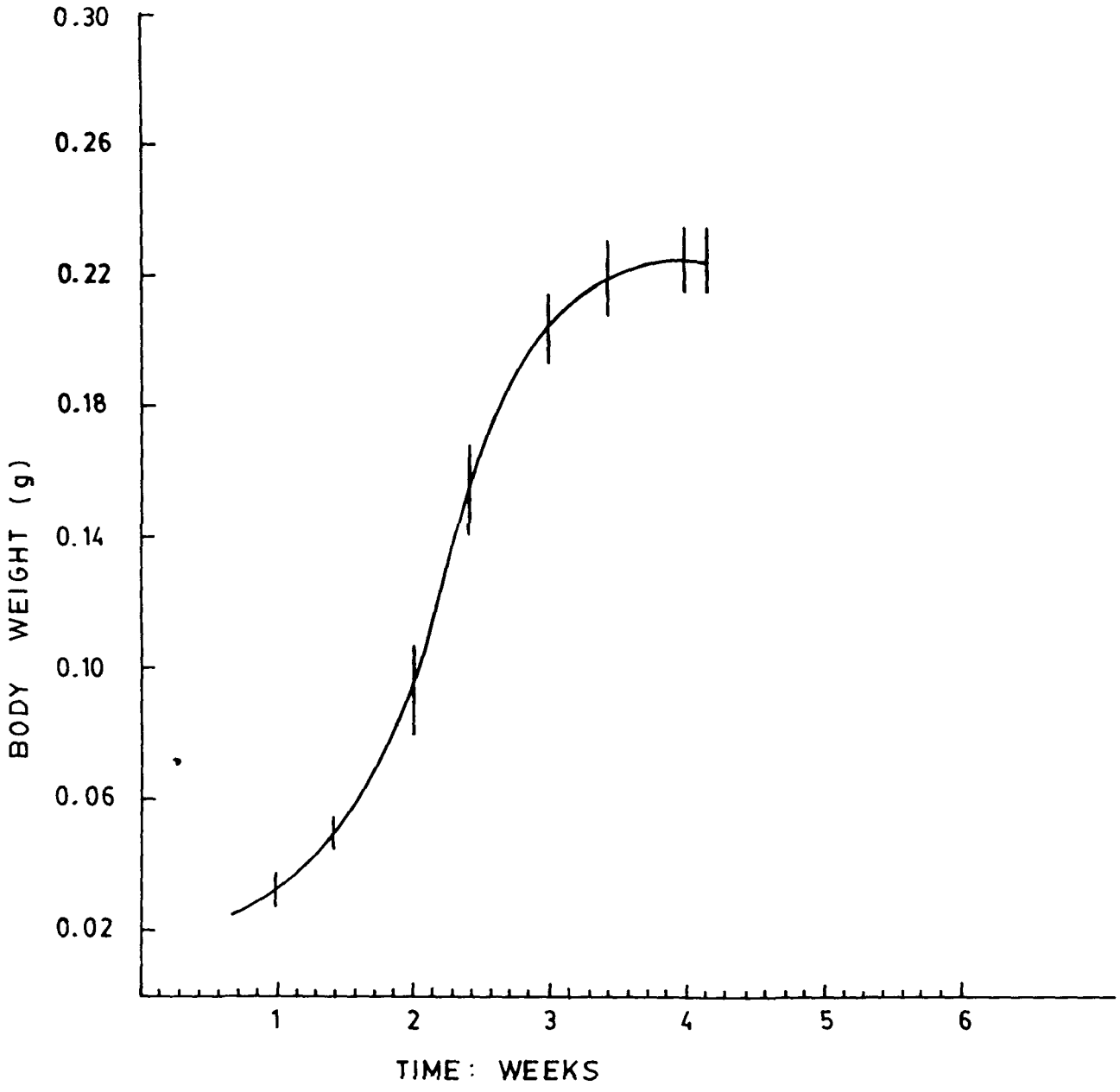


FIG. 4.10 GROWTH RATE OF *Rana limnocharis* TADPOLES REARED IN ISOLATION.

decrease in mean weight at the time when their tail was resorbed. At the completion of metamorphosis on 36th or 37th day, the mean weight of the froglets was 0.170 g.

The curve showing the growth rate is sigmoid. Although the tadpoles came from the same spawn, variations were noted in their mean weight during development (Fig. 4.10). Genetic or chance environmental differences seem to account for such variations. The data of this control experiment represents the null case of no density effect as they were reared individually.

4.4.2 Growth and metamorphosis of tadpoles reared at different densities

Newly hatched tadpoles of the same age and size (2 d old, av. length 5.75 mm), with a mean weight of 0.025 g were randomly selected. They were seperated at 7 densities of 4,8,16,32,64,128 and 256 tadpoles per tray, to see the effect of density on the growth and metamorphosis. The growth rate has been illustrated in a graph (Fig. 4.11). Visual inspection of the growth curves (Fig. 4.11) shows that as the initial density is increased the S-shaped growth curves tend to shift towards the right hand side and also attain a lower body weight. Another inference which can be drawn from the analysis is that as the initial density is increased, the individuals tend to grow at a slower rate and metamorphose at a later date.

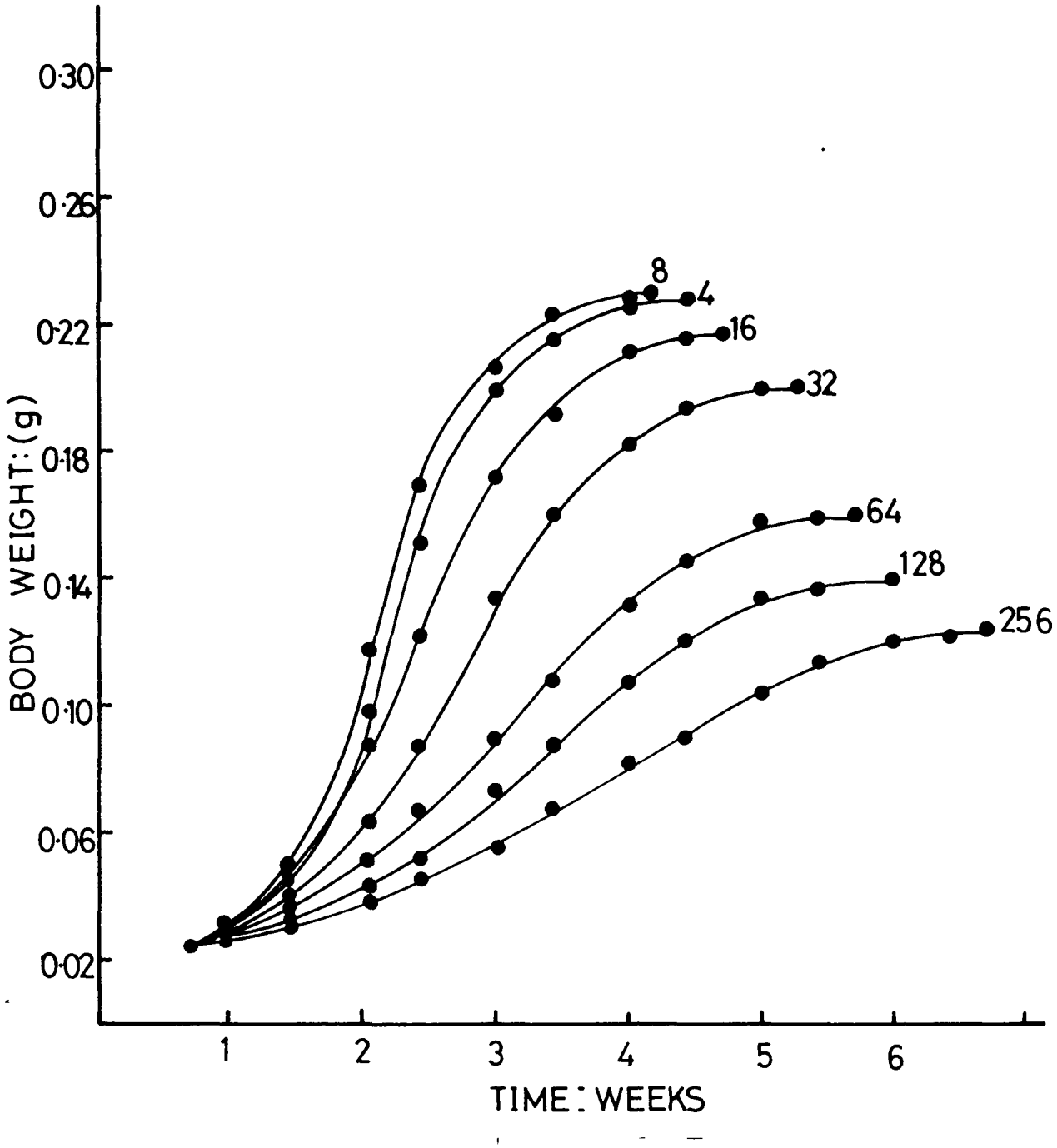


FIG. 4.11 DENSITY DEPENDENT GROWTH OF *Rana limnocharis* TADPOLES. (NUMBERS INDICATE DENSITIES).

Analysis of variance

A one way analysis of variance was used to determine the effect of density on the growth and metamorphosis of tadpoles.

Growth

The weight of the tadpoles in different density treatments were calculated on the 24th day of growth, the time when maximum growth had been attained. The mean weight, range and SD of the tadpoles are compiled in Table 4.16. The analysis of variance (Table 4.17) shows that the calculated value of F (15.06) is greater than the critical value of F at 0.01 level of significance. Hence there is a significant difference in growth rate of tadpoles reared at different densities.

Metamorphosis

The weight of the tadpoles of all density treatments were recorded at the metamorphic climax stage. The range, mean and SD are compiled in Table 4.18. The analysis of variance (Table 4.19) indicates that the calculated value of F (8.18)is greater than the critical value of F at 0.01 level of significance and hence the weight of the tadpoles is significantly affected at metamorphic climax as far as density is concerned.

The growth curves (Fig. 4.11), statistical analysis of growth rate (Table 4.17) and body weight at metamorphic climax (Table 4.19) indicates that density influences the growth rate

TABLE - 4.16 MEAN WEIGHT OF *Rana limnocharis* TADPOLES AFTER 24 DAYS OF GROWTH AS A FUNCTION OF DENSITY.

DENSITY	BODY WEIGHT (g)		± SD
	RANGE	MEAN	
4	0.190-0.258	0.216	0.037
8	0.195-0.260	0.225	0.033
16	0.162-0.214	0.192	0.027
32	0.142-0.190	0.160	0.026
64	0.082-0.135	0.108	0.027
128	0.060-0.106	0.088	0.025
256	0.045-0.090	0.068	0.023

Mean Weight of tadpoles at stage 22 = 0.025 g

TABLE 4.17 - ONE WAY ANALYSIS OF VARIANCE OF MEAN BODY WEIGHT (g) OF *Rana limnocharis* TADPOLES AFTER 24 DAYS OF GROWTH AS A FUNCTION OF DENSITY.

REPLICATES	DENSITY (a = 7)						
	4	8	16	32	64	128	256
1.	0.190	0.220	0.214	0.150	0.082	0.106	0.069
2.	0.258	0.195	0.200	0.142	0.135	0.060	0.090
3.	0.200	0.260	0.162	0.190	0.107	0.098	0.045

Computations

a) Grand total, T = 3.173
b) Correction factor, T^2/N = 0.479425
c) Total sum of squares of all items = 0.563217
d) Total sum of squares (SST) = 0.083792
e) Sum of squares between groups (SSC) = 0.072547
f) Sum of squares within groups (SSE) = 0.011245

Calculation of F-Ratio - ANOVA table

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F-Ratio
Between groups (treatments)	0.072547	6	0.012091	15.06*
Within groups (error)	0.011245	14	0.000803	
Total	0.083792	20		

*F significant at 0.01 level.

TABLE - 4.18 MEAN BODY WEIGHT OF *Rana limnocharis* TADPOLES AT METAMORPHOIC CLIMAX AS A FUNCTION OF DENSITY

DENSITY	BODY WEIGHT (g)		± SD
	RANGE	MEAN	
4	0.194-0.258	0.228	0.032
8	0.198-0.260	0.230	0.031
16	0.190-0.242	0.217	0.026
32	0.180-0.232	0.200	0.028
64	0.140-0.185	0.160	0.123
128	0.114-0.158	0.140	0.023
256	0.104-0.145	0.124	0.021

TABLE - 4.19 ONE WAY ANALYSIS OF VARIANCE OF MEAN BODY WEIGHT(g) OF *Rana limnocharis* TADPOLES AT METAMORPHIC CLIMAX AS A FUNCTION OF DENSITY.

REPLICATES	DENSITY (a = 7)						
	4	8	16	32	64	128	256
1	0.194	0.234	0.242	0.232	0.140	0.158	0.104
2	0.258	0.198	0.219	0.180	0.155	0.114	0.123
3	0.232	0.260	0.190	0.188	0.185	0.148	0.145

Computations

a) Grand total, T = 3.899
b) Correction factor, T^2/N = 0.723914
c) Total sum of squares of all items = 0.768461
d) Total sum of squares (SST) = 0.044547
e) Sum of squares between groups (SSC) = 0.034654
f) Sum of squares within groups(SSE) = 0.009893

Calculation of F-Ratio - ANOVA table

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F-Ratio
Between groups (treatments)	0.034654	6	0.005776	8.18*
Within groups (error)	0.009893	14	0.000706	
Total	0.044547	20		

*F significant at 0.01 level.

and hence reflected at metamorphic climax.

Wilbur and Collins (1973) have suggested that metamorphosis will be initiated only after a minimum threshold size is attained. In the present experiment it was observed that metamorphosis was initiated only after a minimum weight of 0.140g was attained. The individuals at low densities such as 4 &8 grow faster and attain a mean weight of 0.228 g and 0.230 g at metamorphic climax, where as individuals at high densities such as 128 grow slowly and attain a mean weight of 0.140 g and metamorphose by 46 to 47 days. At very high densities such as 256 growth rate is severly affected and the tadpoles showed no sign of metamorphosis till 56 days at which time the experiment was terminated. The mean weight recorded at this time was 0.124 g. Thus the results indicate that a minimum thereshold size of 0.140 g is required for metamorphosis.

4.4.3 Effect of culture medium on growth and metamorphosis of tadpoles

In the present experiment, in one set of the replicates the culture medium was regularly changed twice a week, where as in the other set of replicates water was not changed at all. In this case fresh water was regularly added to replace the water lost by evaporation. Sufficient food (boiled cabbage) was always made available to the tadpoles. The mean weight of the growing tadpoles was recorded twice a week. The values have been plotted in a graph (Fig. 4.12).

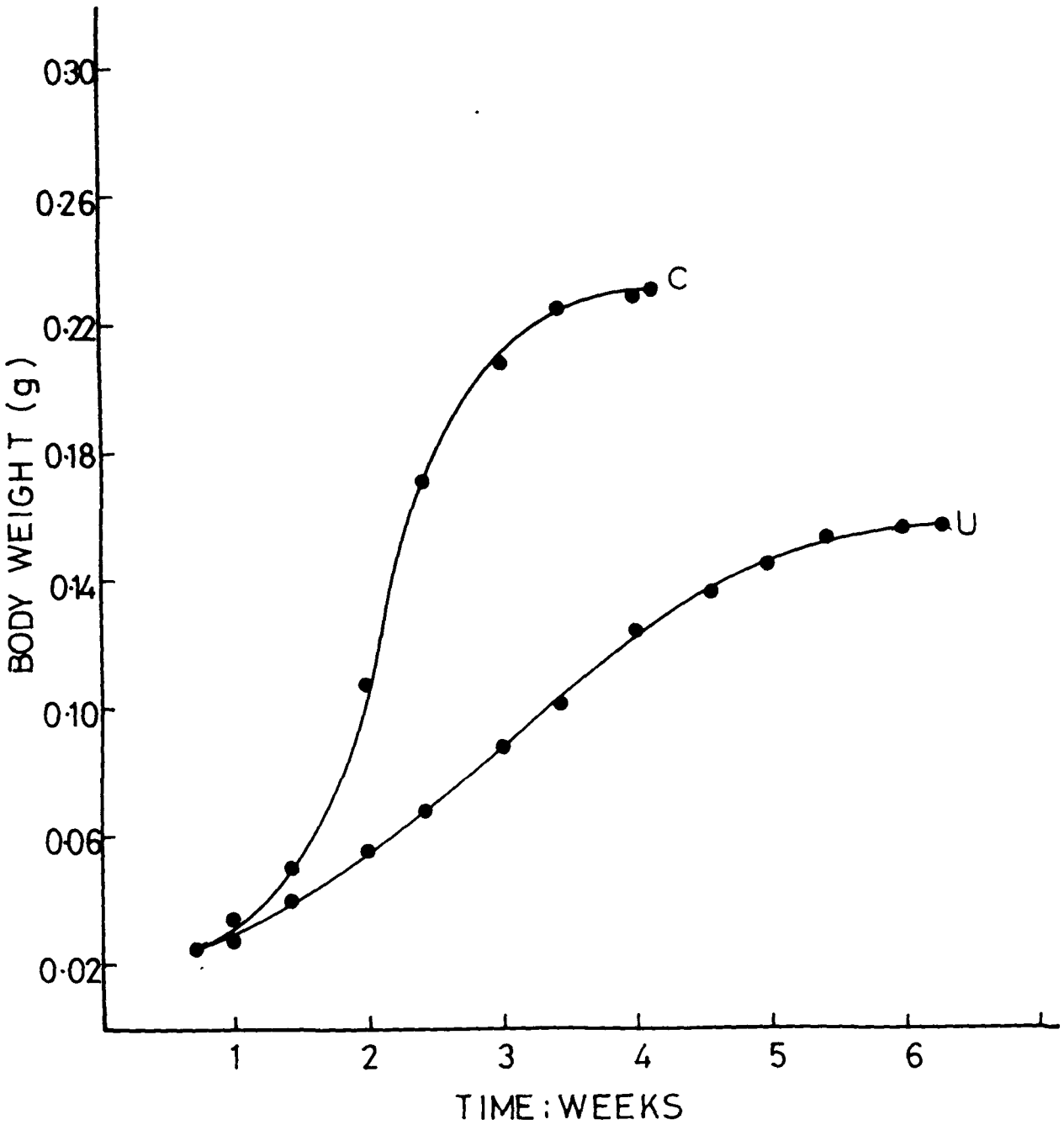


FIG. 4.12 EFFECT OF CULTURE MEDIUM ON GROWTH OF *Rana limnocharis* TADPOLES.

(C-MEDIUM CHANGED REGULARLY, U-MEDIUM NOT CHANGED).

Visual inspection of the graph indicates that the group in which water was regularly changed showed a faster rate of growth. These tadpoles attained a mean weight of 0.0230g (0.196 g- 0 160g) at metamorphic climax and metamorphosed by 36 to 37 days. The other group of tadpoles showed a slower rate of growth. They attained a mean weight of 0.156 g (0.130 g - 0.180 g) at metamorphic climax and metamorphosed by 50 to 52 days.

Analysis of Variance

A one way analysis of variance has been applied to determine the effect of culture medium on growth and metamorphosis of tadpoles.

Growth

The analysis of variance (table 4.21) calculated from the data compiled in table 4.20 indicates that the calculated value of F (23.2) is greater than the critical value of F (21.2) at 0.01 level of significance. Hence there is a significant difference in growth rates of the tadpoles in the two experimental sets.

Metamorphosis

The analysis of variance (table 4.23) calculated from the data compiled in Table 4.22 indicates that the calculated value of F (12.50) is greater than the critical value of F (7.71) at 0.05 level of significance. Hence there is a significant difference in the mean body weight of the tadpoles at metamorphic climax in the two experimental sets.

TABLE - 4.2b MEAN BODY WEIGHT OF *Rana limnocharis* TADPOLES AFTER 24 DAYS OF GROWTH AS A FUNCTION OF CULTURE MEDIUM.

Culture medium	Body weight (g)		± SD
	Range	Mean	
Changed regularly	0.194-0.260	0.224	0.033
Not changed	0.080-0.134	0.102	0.028

Mean weight of tadpoles at stage 22 = 0.025 g.

TABLE - 4.21 ONE WAY ANALYSIS OF VARIANCE OF THE MEAN WEIGHT (g) OF *Rana limnocharis* TADPOLES AS A FUNCTION OF CULTURE MEDIUM.

REPLICATES	TREATMENTS, (a = 2)	
	CULTURE MEDIUM CHANGED REGULARLY	CULTURE MEDIUM NOT CHANGED
1	0.194	0.080
2	0.218	0.092
3	0.260	0.134

Computations

a) Grand total, T = 0.978

b) Correction factor, T^2/N = 0.159414

c) Total sum of squares of all items = 18558

d) Toal sum of squares (SST) = 0.026166

e) Sum of squares between groups (SSC) = 0.022320

f) Sum of squares within groups (SSE) = 0.00384

Calculation of F - Ratio - ANOVA table

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F-Ratio
Between groups (treatments)	0.022326	1	0.022326	23.2*
Within groups (Error)	0.00384	4	0.00096	
TOTAL	0.026166	5		

*F Significant at 0.01 level

TABLE - 4.22 MEAN BODY WEIGHT OF *Rana limnocharis* TADPOLES AT METAMORPHOIC CLIMAX AS A FUNCTION OF CULTURE MEDIUM.

Culture medium	Body weight (g)		± SD
	Range	Mean	
Changed regularly	0.196-0.260	0.230	0.031
Not Changed	0.144-0.178	0.156	0.18

TABLE - 4.23 ONE WAY ANALYSIS OF VARIANCE OF THE MEAN WEIGHT (G) OF *Rana limnocharis* TADPOLES AT METAMORPHIC CLIMAX AS A FUNCTION OF CULTURE MEDIUM.

	CULTURE MEDIUM (a = 22)	
REPLICATES	CHANGED REGULARLY	NOT CHANGED
1	0.260	0.144
2	0.198	0.178
3	0.234	0.148

COMPUTATIONS

a) Grand Total, T = 1.162
b) Correction factor, T^2/N = 0.225041
c) Total sum of squares of all items = 0.235884
d) Total sum of squares (SST) = 0.010843
e) Sum of squares between groups (SSC) = 0.008213
f) Sum of squares within groups (SSE) = 0.00263

CALCULATION OF F-RATIO - ANOVA TABLE

SOURCE OF VARIATION	SUM OF SQUARES	DEGREE OF FREEDOM	MEAN SQUARES	F-RATIO
BETWEEN GROUPS (treatments)	0.008213	1	0.008213	12.50*
WITHIN GROUPS (error)	0.00263	4	0.000657	
TOTAL	0.010843	5		

*F Significant at 0.05 level

The curves (Fig 4.12), statistical analysis of growth rate (Table 4.21) and body weight at metamorphic climax (Table 4.23) indicates that culture medium infulences the growth rate and hence reflected at metamorphic climax. When the culture medium of the tadpoles was regularly changed it resulted in a better growth rate and faster rate of metamorphosis than those in which culture medium was not changed.

4.5 EXPERIMENTS IN RELATION TO TEMPERATURE

4.5.1. Effect of temperature on the development of tadpoles

The present experiment was conducted to see the effect of temperature on growth and metamorphosis of *Rana limnocharis* tadpoles hatched in the laboratory. Two sets of 20 tadpoles each were reared at constant temperatures of 10° C and 32° C respectively. The third set was reared at room temperature ($\bar{x}$ 23°C) as a control. The mean weight of growing tadpoles recorded twice a week has been plotted in a graph (Fig 4.13).

The tadpoles reared at room temperature attain a mean body weight of 0.230 g (0.198 g - 0.260 g) at metamorphic climax and metamorphose by 36 to 37 days.

Tadpoles reared at high temperature of 32° C attain a lower body weight of 0.180 g and metamorphose by 34 to 35 days.

Tadpoles reared at low temperature of 10°C did not metamorphose till 56 days at which time the experiment was terminated. The mean weight recorded at that time was 0.280g.

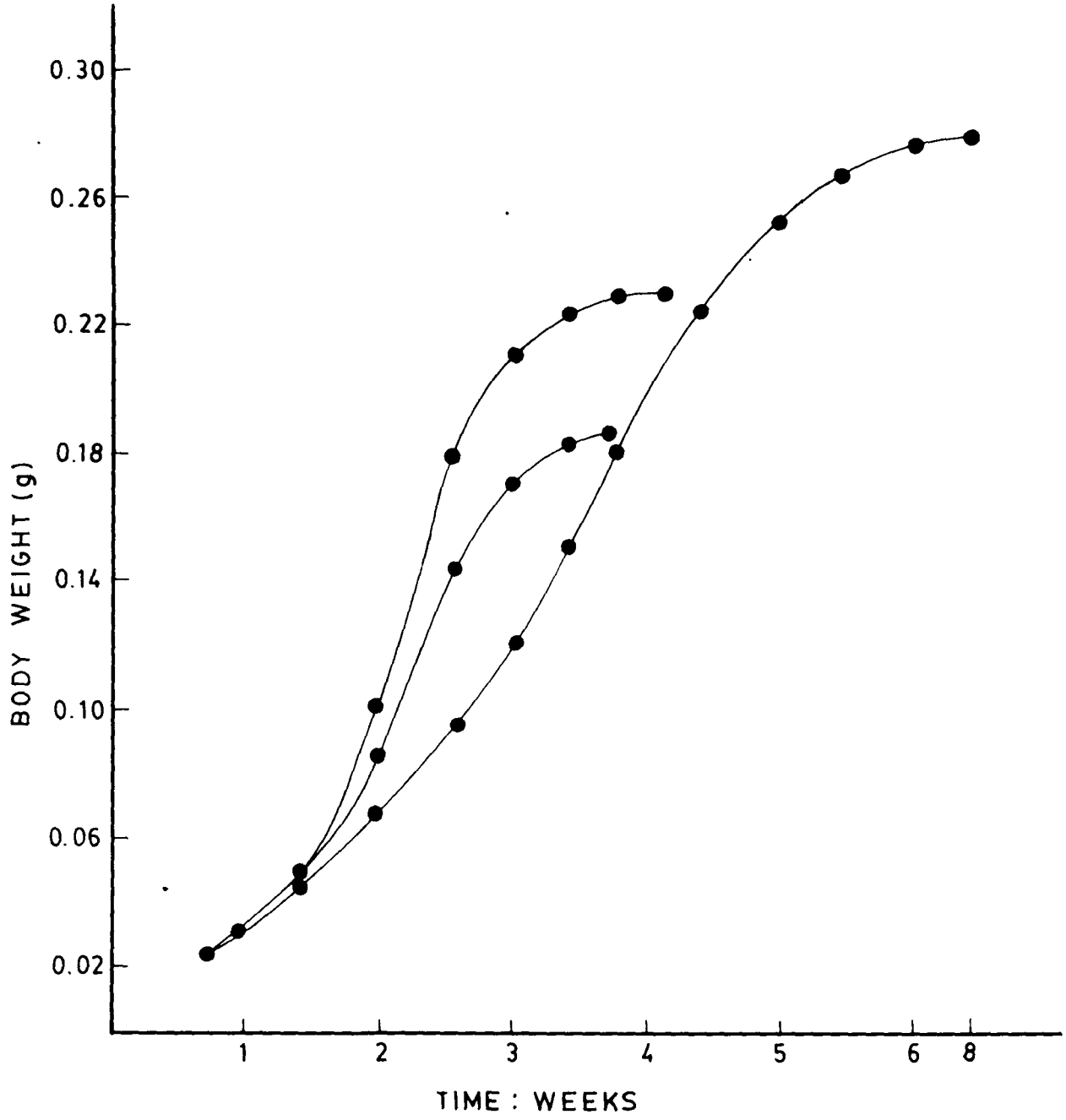


FIG. 4.13 EFFECT OF TEMPERATURE ON GROWTH OF *Rana limnocharis* TADPOLES.

Analysis of Variance

A one way analysis of variance was used to determine the effect of temperature on the rate of growth and metamorphosis of tadpoles.

Growth

The weight of the tadpoles of all the treatments were recorded on the 24th day of growth, the time when maximum growth had been attained. The mean weight, range and SD of the tadpoles are compiled in Table 4.24. The analysis of variance (Table 4.25) shows that the calculated value of F (3.96) is greater than the critical value of F at 0.01 level of significance. Hence there is a significant difference in growth rates of tadpoles reared at the three different temperatures.

Metamorphosis

The weight of the tadpoles of all the treatments were recorded at the metamorphic climax. The range, mean and SD are compiled in Table 4.26. The analysis of variance (Table 4.27) shows that the calculated value of F (12.37) is greater than the critical value of F at 0.01 level of significance. Hence there is a significant difference in the mean weight attained by the tadpoles at metamorphic climax, when they are reared at three different temperatures.

TABLE - 4.24 : MEAN BODY WEIGHT OF *Rana limnocharis* TADPOLES AFTER 24 DAYS OF GROWTH AS A FUNCTION OF TEMPERATURE.

TEMPERATURE ^{o}C	BODY WEIGHT (g)		± SD.
	RANGE	MEAN	
10	0.130 - 0.180	0.154	0.025
23	0.154 - 0.208	0.186	0.026
32	0.200 - 0.246	0.224	0.023

* Mean weight of tadpoles at stage 22 = 0.025 g.

TABLE 4.25 ONE WAY ANALYSIS OF VARIANCE OF THE MEAN WEIGHT OF *Rana limnocharis* AS A FUNCTION OF TEMPERATURE AFTER 24 DAYS OF GROWTH

	TEMPERATURE °C (a=3)		
REPLICATES	10	23	32
1	0.130	0.154	0.200
2	0.180	0.196	0.200
3	0.152	0.208	0.246

COMPUTATIONS

a) Grand Total, T = 1.6660
b) Correction Factor, T^2/N = 0.308395
c) Total sum of squares of all items = 0.318316
d) Total sum of squares (SST) = 0.009921
e) Sum of squares between groups (SSC) = 0.005646
f) Sum of squares within groups (SSE) = 0.004275

CALCULATION OF F-RATIO - ANOVA TABLE

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F-RATIO
BETWEEN GROUPS (treatments	0.005646	2	0.002823	3.96*
WITHIN GROUPS (error)	0.004275	6	0.0007125	
TOTAL	0.00992	8		

* F Significant at 0.01 level.

TABLE - 4.26 MEAN BODY WEIGHT OF *Rana limnocharis* TADPOLES AT METAMORPHIC CLIMAX AS A FUNCTION OF TEMPERATURE.

TEMPERATURE ^{o}C	BODY WEIGHT (g)		$\pm$ SD
	RANGE	MEAN	
10	0.268 - 0.340	0.280	0.037
23	0.198 - 0.260	0.230	0.031
32	0.160 - 0.196	0.180	0.018

TABLE 4.27 ONE WAY ANALYSIS OF VARIANCE OF THE MEAN WEIGHT (g) OF *Rana limnocharis* AS A FUNCTION OF TEMPERATURE

REPLICATES	TEMPERATURE °C (a=3)		
	10	23	32
1.	0.268	0.232	0.196
2.	0.292	0.198	0.160
3.	0.340	0.266	0.184

COMPUTATIONS

a)	Grand Total, T	= 2.130
b)	Correction Factor, T^2/N	= 0.5041
c)	Total sum of squares of all items	= 0.531188
d)	Total sum of squares (SST)	= 0.027088
e)	Sum of squares between gruups (SSC)	= 0.0218
g)	sum of squares within groups (SSE)	= 0.005288

CALCULATION OF F-RATIO - ANOVA TABLE

SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEANS SQUARES	F-RATIO
BETWEEN GROUPS (treatments)	0.0218	2	0.0109	12.37*
WITHIN GROUPS (error)	0.005288	6	0.000881	
TOTAL	0.027088	8		

*F Significant at 0.01 level.

The growth curves (Fig 4.13), statistical analysis of growth rate (Table 4.25) and body weight at metamorphic climax (Table 4.27) indicates that temperature influences the growth rate of the tadpoles and hence reflected at metamorphic climax. High temperature results in a lower body weight at metamorphic climax as compared to those reared at room temperature. However there was only a slight variation observed in the timing of metamorphosis in the two sets. In case of tadpoles reared at low temperature growth rate was slow and they attained a larger body weight and did not metamorphose till 56 days.

4.5.2 Estimation of thermal tolerance levels

i) Critical thermal maxima (CTMax)

a) Tadpoles

The tadpoles were selected at stages 26-27. One tadpole was introduced into the test beaker for each experiment. The temperature of the water in the test beaker at the start of each experiment was 22.5^{o}C (room temperature). It swam up and down or rested at the bottom of the beaker. When the water temperature was raised from 23°C to 30°C, there were no apparent changes in their movements and behaviour. On further increase in water temperature at the rate of 1°C/min, occassional short fast movements were observed. These movements became pronounced at 35°C to 38°C. At 38°C to 40°C tadpoles started moving faster

and the duration of their active movements also increased. At 40°C a notable change observed was loss of coordinated movements of the tadpoles and disoriented swimming on their sides. When the tadpoles approached the CTMax end point, they showed violent spasmodic thrashings of the tail as well as the body. At the CTMax end point the tadpoles went down sinking to the bottom and did not respond to mechanical stimulation with forceps. As soon as this behaviour was seen, the temperature was noted and the heating was stopped. The test beaker was placed at room temperature to cool down for the tadpole to recover.

The data has been presented in table 4.28. The upper thermal tolerance level (CTMax) was found to be 41.5°C (40.5°C - 42.2°C).

b) Juveniles

The procedure for determining the CTMax of juveniles was similar to that of the tadpoles. The juveniles of Snout-Vent length 30 mm had a mean CTMax of 40.5°C (40.0°C - 40.8°C).

ii) Critical thermal minima (CTMin)

a) Tadpoles

The lower thermal tolerance level (CTMin) of tadpoles of stages 26-27 was determined in a similar way, except that the water

TABLE - 4.28 THERMAL TOLERANCE LEVELS(CTMax AND CTMin) OF TADPOLES AND JUVENILES OF *Rana limnocharis*.

THERMAL TOLERANCE LEVELS	DEVELOPMENTAL STAGES	RANGE	MEAN	± SD
CTMax °C	TADPOLES STAGES 26-27	40.5-42.2	41.5	0.62
	JUVENILES SVL 28-30 mm	40.0-40.8	40.5	0.30
CTMin °C	TADPOLES STAGES 26-27	5.0- 6.6	5.9	0.54
	Juveniles SVL 28-30 mm	9.0-10.0	9.6	0.32

in the test beaker was cooled at the rate of 1°C/min. For each experiment one tadpole was introduced into the test beaker containing water (room temp 23°C). It swam up and down or rested at the bottom of the beaker. As the temperature of the water was reduced, the movements of the tadpoles was also reduced. It was comparatively difficult to determine the CTMin, than CTMax as the movements of tadpoles greatly reduced at low temperature.

The data has been presented in table 4.28. The CTMin of tadpoles was found to be 5.9°C with a range of 5.0°C to 6.6°C.

b) Juveniles

The procedure used for CTMin determination of juveniles was similar to that used for tadpole stages. The CTMin of juveniles of Snout-Vent length 30 mm was 9.6°C (9°C to 10°C).

4.5.3) Analysis of the effect of thermal acclimation on CTMax

In order to determine the effect of high and low acclimation on CTMax, the tadpoles were selected at stages 26-27 and acclimated for a period of four days at constant temperatures of 10.0°C and 35.0°C. A set of 12 tadpoles were maintained at room temperature (23.0°C) as a control. The CTMax of both categories of tadpoles was determined. The data has been presented in Table 4.29.

TABLE 4.29 CTMax OF *Rana limnocharis* TADPOLES (STAGE 26-27) ACCLIMATED AT DIFFERENT TEMPERATURES FOR A PERIOD OF FOUR DAYS.

CTMax ^{o}C	ACCLIMATION TEMPERATURE ^{o}C		
	10.0	23 (RT).	35.0
Mean	37.2	41.5	42.1
Range	36.5 - 38.0	10.5 - 42.2	41.5 - 42.5
SD	0.51	0.59	0.39

The tadpoles acclimated at 10°C have a mean CTMax of 36.8°C and a range of 36.5°C to 37.5°C. The tadpoles acclimated at 35.0°C have a mean CTMax of 42.1°C and a range of 40.3°C to 42.0°C while those maintained at room temperature had a mean CTMax of 41.5°C and a range of 40.5°C to 42.2°C.

The experiment indicated that the tadpoles acclimated at low temperatures (10 .0°C) have a low CTMax, those acclimated at room temperature (23.0°C) hava a high CTMax and those acclimated at high temperature (35.0°C) have a still higher CTMax. Thus the tadpoles are able to regulate their CTMax with the temperature at which they have been acclimated.

Rise in temperature and CTMax

In order to determine whether rise in temperature of the medium had any effect on the CTMax of tadpoles of different sizes, the effect of three different rates of 1°C/minute, 1°C/5 minutes and 1°C/10 minutes was examined.

For the sake of convenience the tadpoles in each of the sets of experiment were grouped into two size classes i) Small tadpolesof stages 23-27 ii) Large tadpoles of stages 28-29. The CTMax was determined and the data obtained has been presented in Fig. 4.14.

Effect on younger tadpoles

The small tadpoles which were subjected to rise in water temperature at the rate of 1°C/minute had a mean CTMax of 41.5°C and a range of 40.3°C to 42.0°C.

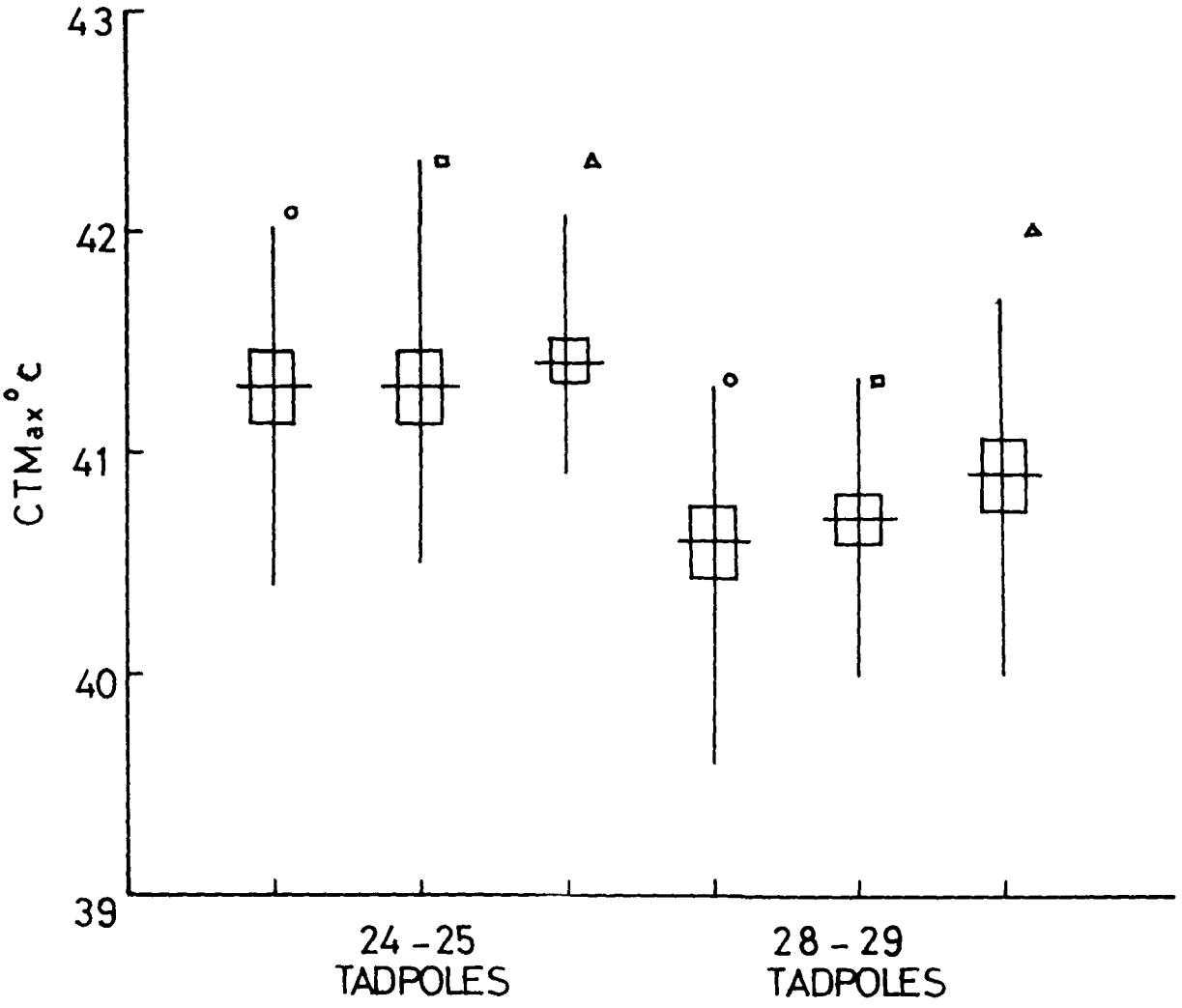


FIG. 4.14 EFFECT OF HEATING RATE ON CTMax OF TADPOLES OF *Rana limnocharis* AT TWO DIFFERENT STAGES OF DEVELOPMENT.

FIGURES WITH DOT INDICATE TADPOLES HEATED AT THE RATE OF 1^{o}C/MINUTE.

FIGURES WITH SQUARES INDICATE TADPOLES HEATED AT THE RATE OF 1^{o}C/5 MINUTES.

FIGURES WITH TRIANGLES INDICATE TADPOLES HEATED AT THE RATE OF 1^{o}C/10 minutes.

Those subjected to rise in water temperature at the rate of 1°C/5 minutes had a mean CTMax of 41.5°C and a range of 40.5°C to 42.3°C.

The tadpoles subjected to a slower rate of increases in water temperature of 1°C/10 minutes had a mean CTMax of 41.6°C and a range of 41.0°C to 42.3°C.

Effect on older tadpoles

The tadpoles of stages 28-29 subjected to a rise in water temperature at the rate of 1°C/minute had a mean CTMax of 40.6°C and a range of 39.3°C to 41.5°C.

Those subjected to a rise in water temperature at the rate of 1°C/5 minutes had a mean CTMax of 40.7°C and a range of 40.0°C to 41.5°C.

The tadpoles subjected to a slower rate of heating of 1°C/10 minutes had a mean CTMax of 40.8°C and a range of 40.0°C to 42.0°C.

The experiment indicates that heating rate does not effect the CTMax of smaller tadpoles (Stages 22-27), but it does have an effect on larger tadpoles (stages 28-29).

4.5.4 Analysis of CTMax at different developmental stages

i) Embryonic stages

For this experiment, the CTMax of 4 different embryonic

stages namely stage 4 (four cell stage), stage 11 (blastopore stage), stage 17 (tail bud stage) and stage 22 (tail fin circulation stage) were used. Groups of 12 embryos of each stage were placed in beakerscontaining 100 ml of water and for each stage there were 3 replicates. The beakers were placed in constant water baths maintained at 3 different temperatures of 40°C, 42°C and 43°C.

As the water temperature of the beakers rose, the embryos moved towards the water surface as numerous bubbles were observed on the surface of the jelly covers. The bubbles were removed by gently shaking the embryos. They were exposed to constant temprratures of 40°C, 42°C and 43°C for a period of 2 hrs and then the beakers were allowed to cool down to room temperature. After 24 hrs the number of dead embryos in each replicate were counted and percentage mortality calculated. Critical temperature at which half of the embryos died was recorded.

The data has been illustrated in Fig..4.15A. The experiment shows that at all embryonic stages the mortality was almost 100% at 43°C and at stage 4 at 42°C also the mortality was 100%, but it reduced sharply to about 8.5% at stage 11. It remained so till stage 17 and it increased to 46.7% at stage 22.

In the case of the embryos exposed to 40°C temperature, a mortality of about 53.5% was observed at stage 4, while it reduced to almost 3% at stage 11 and remained so till stage 17.

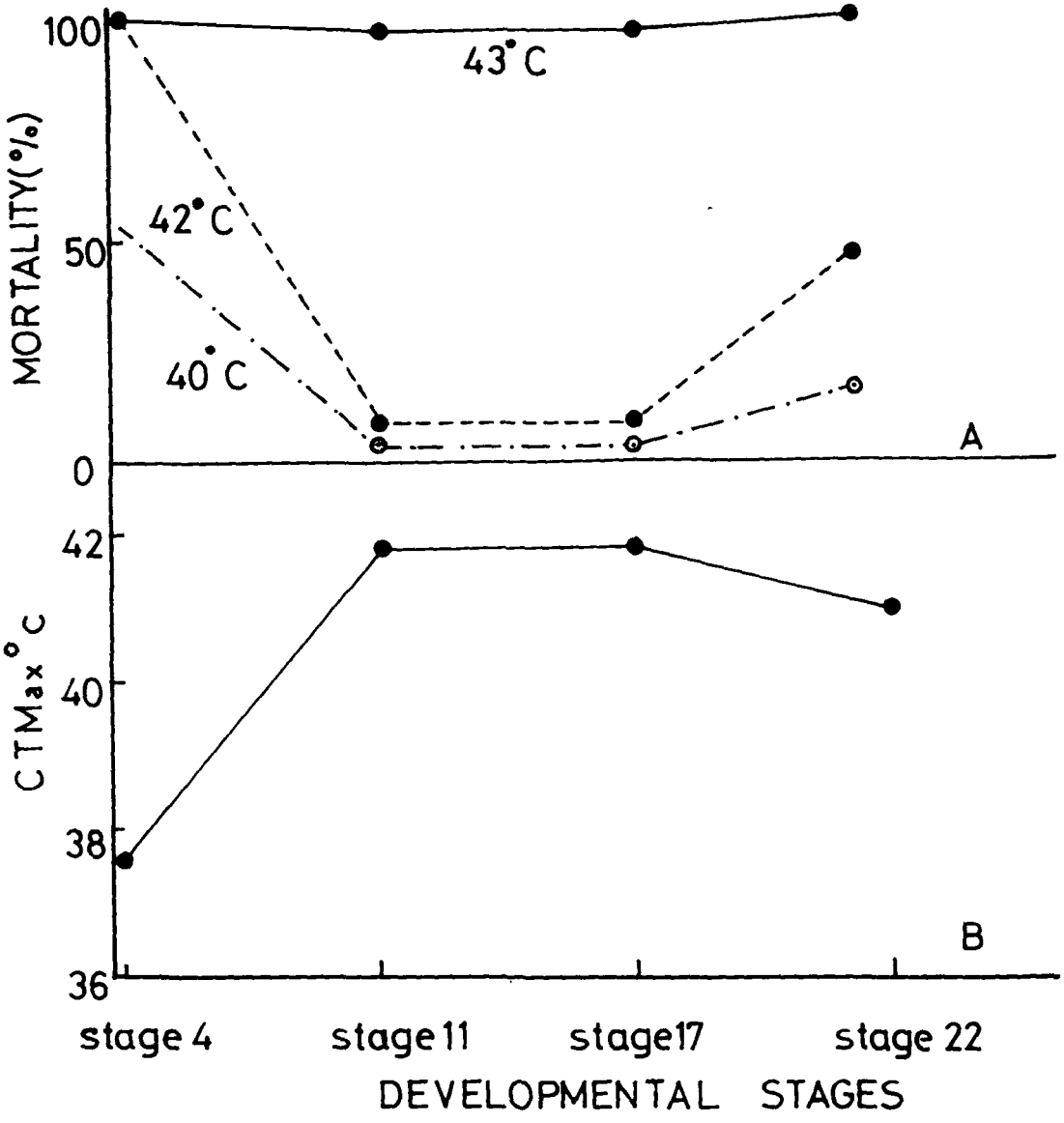


FIG. 4.15 PERCENTAGE MORTALITY (A) AND CRITICAL THERMAL MAXIMA (B) AT DIFFERENT EMBRYONIC STAGES OF *Rana limnocharis* EXPOSED TO VARIOUS TEST TEMPERATURES FOR 2 HR.

At stage 22 there was again a slight increase in mortality to 16.5%.

There was also a distinct pattern of changes in the CTMax during the embryonic development (Fig 4.15B). At stage 4 the CTMax was about 37.6°C and it rose sharply to 41.8°C at stage 11. At stage 17 also the CTMax recorded was 41.8°C, while at stage 22, it decreased to 41.1°C.

ii) Tadpole and Juvenile stages

The following two aspetcs of thermal tolerance of tadpoles have been examined.

a) Response to rise in water temperature.

b) CTMax at different developmental stages.

a) Response to rise in water temperature

In the present experiment, the response of different developmental stages to rise in water temperature has been examined with a view to see if different stages respond in a different way or not.

Stages wise behaviour and response of the tadpoles to rise in water temperature has been tabulated in table 4.30.

The experimental data shows that different developmental stages respond to rise in water temperature in a different way. Tadpoles of stages 23-25 and stages 26-27 which had a comparatively

TABLE 4.30 *Rana limnocharis* : RESPONSE OF DEVELOPMENTAL STAGES TO RISE IN WATER TEMPERATURE

WATER TEMP°C	TADPOLE STAGES					JUVENILES (mm)		
	23-25	26-27	28-29	30-31	32	16-18	22-24	28-30
22.5	n	n	n	n	n	n	n	n
27.0	+	+	+	+	+	+	+	+
31.0	+	+	+	-	o	-	-	+
35.0	+	+	+	-	@	o	o	+
37.0	-	-	-	o	*	@	o	-
38.5	-	-	-	#		*	@	-
39.0	-	-	o	@			*	o
39.5	o	o	o	*				o
40.0	o	o	#					@
40.5	0	0	@					*
40.6	#	o	*					
41.0	@	#						
41.4	*	@						
41.5		*						
42.0								

Starting Temperature = 22.5
n = normal movement,
+ = no remarkable changes observed
- = occasional fast movements
o = movements increased
# = sudden violent jerkings with distinct tail spasms
@ = respond only when mechanically stimulated
* = CTM end point.

higher CTMax showed notable changes in their movements only at high temperature of 39.5°C, whereas tadpoles of stages 30-31 showed changes in behaviour at 37°C itself. Newly metamorphosed froglets showed distinct changes in behavioural movements at 31°C itself. There was an increase in the temperature at which distinct movements were seen in case of post-metamorphic juveniles.

Distinct variations were observed in the time period of recovery required by tadpoles of different stages. This period of recovery varied from 5 to 60 minutes. The tadpoles without limbs (stages 23-27) recovered within 5 to 20 minutes, where as the tadpoles with limbs (stages 28-29) required about 30 to 60 minutes to recover.

b) CTMax at different developmental stages

Based on the experiment described above and the tadpoles and juveniles showing their tolerance levels, they have been grouped into 8 categories.

Category	Stages	CTMax(°C)
1	23-25	41.4
2	26-27	41.5
3	28-29	40.6
4	30-31	39.5
5	32	37.0
6	Juveniles(16-18 mm)	38.5
7	Juveniles(22-24 mm)	39.0
8	Juveniles(28-30 mm)	40.5

Within each category the differences of CTMax was not that remarkable. The pattern of changes of CTMax of different categories has been presented in Fig. 4.16.

The data has been subjected to student's t-test, to examine whether ther is any difference in CTMax's of different categories. The analysis shows that the CTMax of post metamorphic juveniles (40.5^{o}C) was higher than newly metamorphosed froglets (37^{o}C). [Juveniles, 28-30 mm vs stage 32, t = 26.35, df = 22, P < .01]and Juveniles of 16-18 mm size [Juveniles (28-30 mm vs Juveniles (16-18 mm), t = 13.91, df = 22, P < .01] was lower than early larval stages (23-25) [Juveniles (28-30 mm) vs stages23-25, t=5.08, df=22 P<.01 The CTMax of newly metamorphosed froglets was 37^{o}C which was the lowest recorded. (Plate 4.2. A,B,C & D; for description see page 145.)

4.5.4. Changes in CTMin at larval and Juvenile stages

For this experiment tadpoles of two different stages of 23-25 and 26-27 were selected, while the juveniles at snout vent length 16-18 mm and 28-30 mm were selected. The mean CTMin at stages 23-25 was 7.6^{o}C (6.9^{o}C - 8.0^{o}C) and at stages 26-27 was 5.9^{o}C (5.0^{o}C - 6.6^{o}C). The juveniles of snout vent length 16-18 mm had a mean CTMin of 10.0^{o}C (9.4^{o}C - 10.5^{o}C), while those of snout vent length 28-30 mm had a mean CTMin of 9.6^{o}C (9.0^{o}C - 10.0^{o}C). The values have been presented in Fig.(4.17). The results indicate that there is a sharp decline in the mean CTMin from stage 22 to stage 26.

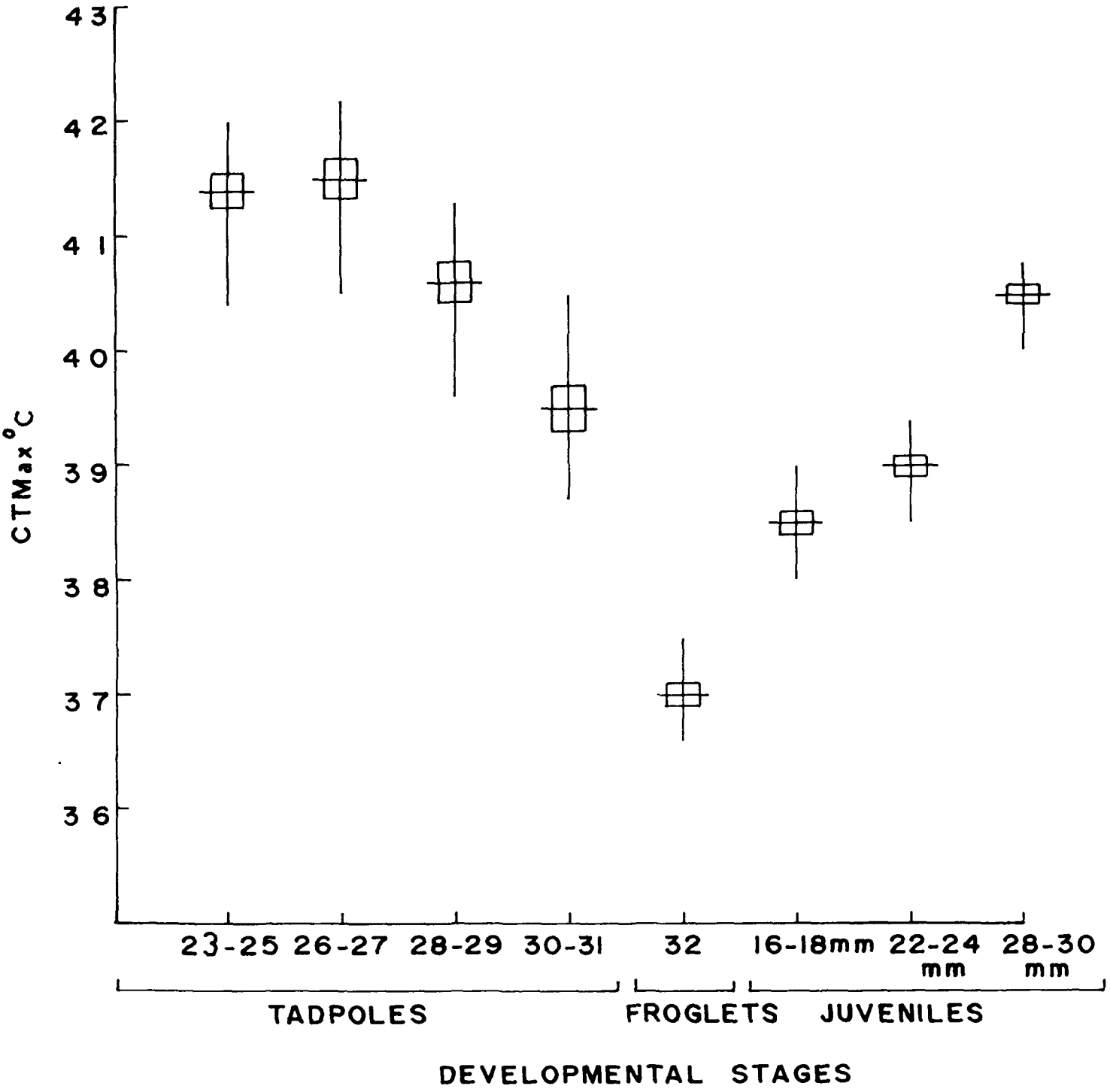


FIG. 4.16 CTMax OF PRE-METAMORPHIC (STAGES 23-29), METAMORPHOSING (STAGES 30-31), FROGLETS (STAGE 32) AND POST-METAMORPHIC (JUVENILES) STAGES OF *Rana limnocharis*.
HORIZONTAL LINES, RECTANGLES AND VERTICLE LINES REPRESENT MEANS, STANDARD ERROR OF THE MEAN AND RANGES RESPECTIVELY. SAMPLE SIZE AT EACH STAGE IS 12.

A

B

PLATE 4.2 A - TADPOLES OF *Rana limnocharis* IN NATURAL HABITAT DURING RAINY SEASON.

B - VIEW OF HABITAT BEING PARTLY DRIED UP.

C

D

PLATE 4.2 C & D CLOSE UP VIEWS, SHOWING TADPOLES OF *Rana limnocharis* ACCUMULATED IN SMALL AREAS WHERE LITTLE WATER IS PRESENT.

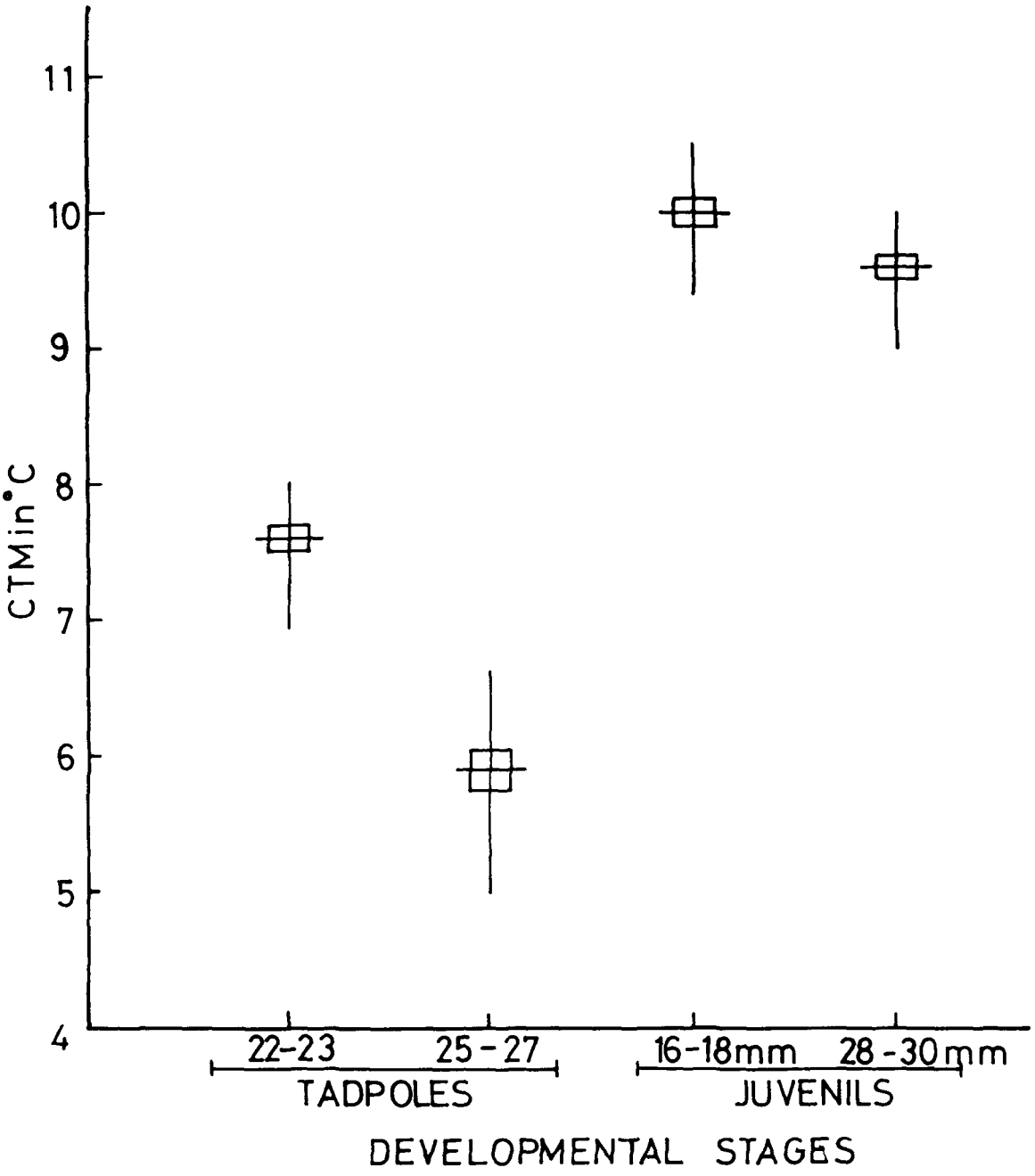


FIG. 4.17 CTMin OF PRE-METAMORPHIC (STAGES 23-27) AND POST-METAMORPHIC (JUVINILES) STAGES OF *Rana limnocharis*. HORIZONTAL LINES, RECTANGLES AND VERTICLE LINES REPRESENT MEANS, ONE STANDARD ERROR OF THE MEAN AND RANGES RESPECTIVELY. SAMPLE SIZE AT EACH STAGE IS 12.

This was followed by sharp increase in mean CTMin to 10.0°C at the juvenile stages of SVL length 16-18 mm The juveniles of 28-30 mm size had a slightly less CTMin of 9.6°C.

4.5.5 Analysis of Daily rhythms of CTMax

With a view to know whether the tadpoles of *Rana limnocharis* possess any variation of the CTMax at different timinings of the day, the following experiment was performed. Side by side oberva-tions on the daily cycles of the behavioural movement of tadpoles of *Rana limnocharis* and *Bufo melanostictus* were made.

The mean CTMax of groups of tadpoles (stages 26-27) were determined at 0500 hr, 0800 hr, 1100 hr, 1400 hr, 1700 and 2000 hr. The data obtained has been presented in Fig. 4.18. It was observed that the mean CTMax was 40.6°C(40.2°C-41.3°C)at 0500 hr and increased sharply to 41.3°C at 0800 hr (40.8°C - 42.2°C). This was followed by slight increase to 41.6°C at 1100 hr (40.8°C - 42.4°C). It remained almost unchanged till 1400 hr when a slight decrease to 41.5°C (40.7°C - 42.1°C) was observed. The mean CTMax then further decreased to 41.0°C (40.3°C - 41.5°C) at 1700 hr and to 40.8°C (40.2°C-41.2°C)at 2000 hr. Thus a temporal pattern of variations in CTMax was observed during the day period.

To determine whether there is any significant difference in different pair combinations of the CTMax's at different timings of the day, all possible pairs of combinations were subjected to

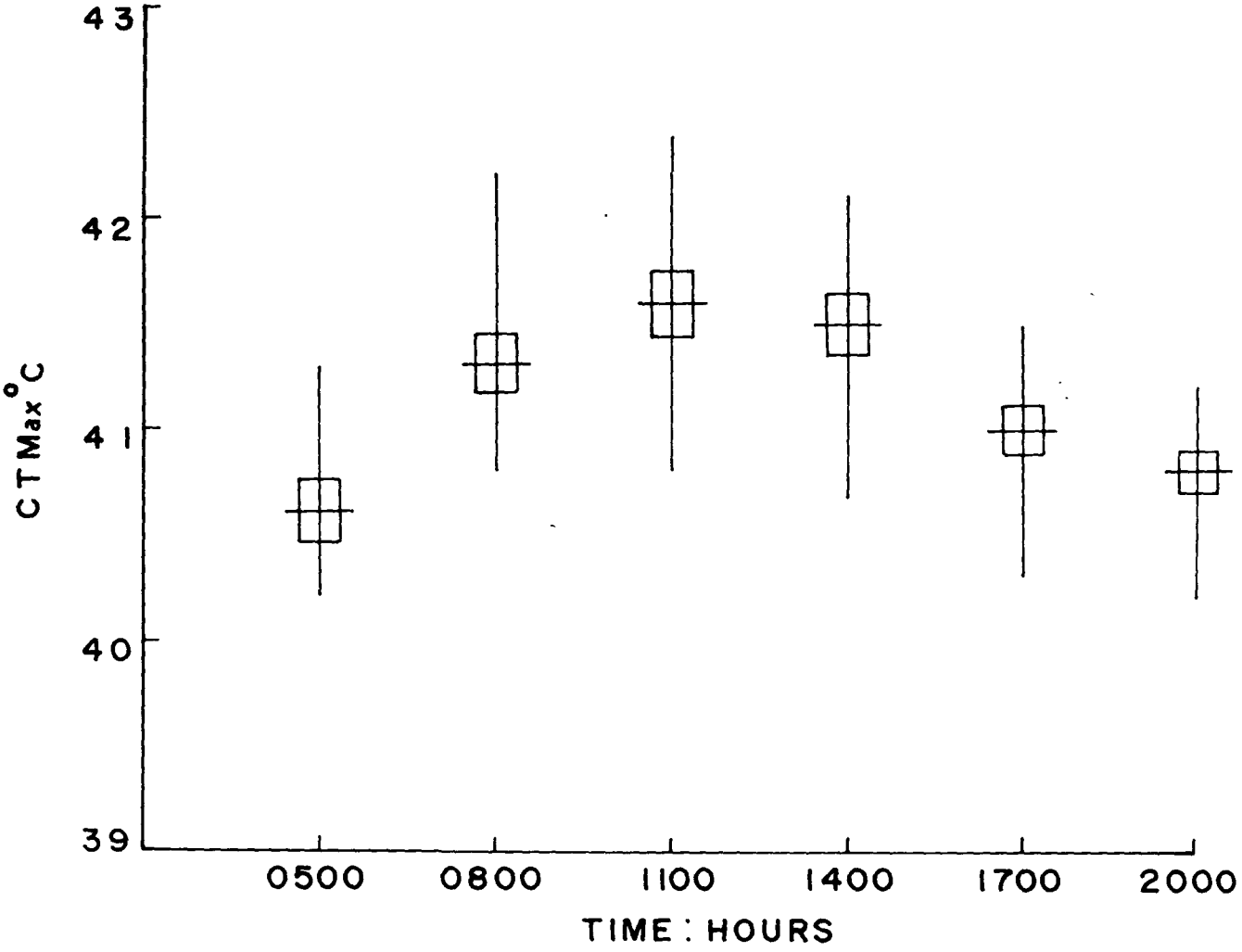


FIG. 4.18 CHANGES IN CTMax OF *Rana limnocharis* TADPOLES (STAGES 26-27) OVER A 15 - HR PERIOD. HORIZONTAL LINES, RECTANGLES AND VERTICLE LINES REPRESENT MEANS, ONE STANDARD ERROR OF THE MEAN AND RANGE RESPECTIVELY. SAMPLE SIZE FOR EACH POINT IS 12.

students t-test. Significant differences were observed in different pair combinations (see Table 4.31), suggesting that there exists a daily rhythm of CTMax.

Observations on the variations in temperature experienced in the field were also noted, as they directly affect the variations in temperature of the water bodies. The variations in temperature during the day are illustrated in Fig. 4.19.

There was a steep increase in temperature from 19.8°C at 0500 hr to 24.0°C at 0800 hr. The temperature remained fairly constant at 29.0°C till 1400 hr when a slight decrease to 28.0°C was noted. This was followed by a further decrease to 25.6°C at 1700 hr and to 22.0°C at 2000 hr.

On comparing the variations in temperature experienced in the field with the variations in CTMax, it is observed that the variations in CTMax appear to anticipate the variations in temperature experienced in the field

DAILY CYCLES OF DISTRIBUTION AND ACTIVITY OF TADPOLES UNDER NATURAL CONDITIONS.

Rana limnocharis : Field observations on the behavioural movements tadpoles of *Rana limnocharis* during the day time when the shallower edges are warmer, and show intense feeding activity, but no distinct aggregation formations were seen.

TABLE 4.31 - MATRIX OF T - VALUES COMPARING THE MEAN CTMax FOR ALL PAIR COMBINATIONS OF *Rana limnocharis* TADPOLES (STAGES 26-27).

TIME (h)	1100	1400	1700	2000	0500
0800	1.51	0.99	1.69	3.03*	4.06*
1100		0.47	3.18*	4.52*	5.45*
1400			2.57+	3.85*	4.78*
1700				1.30	2.48+
2000					1.36

\+ Significantly different at 0.05 level

* Significantly different at 0.01 level

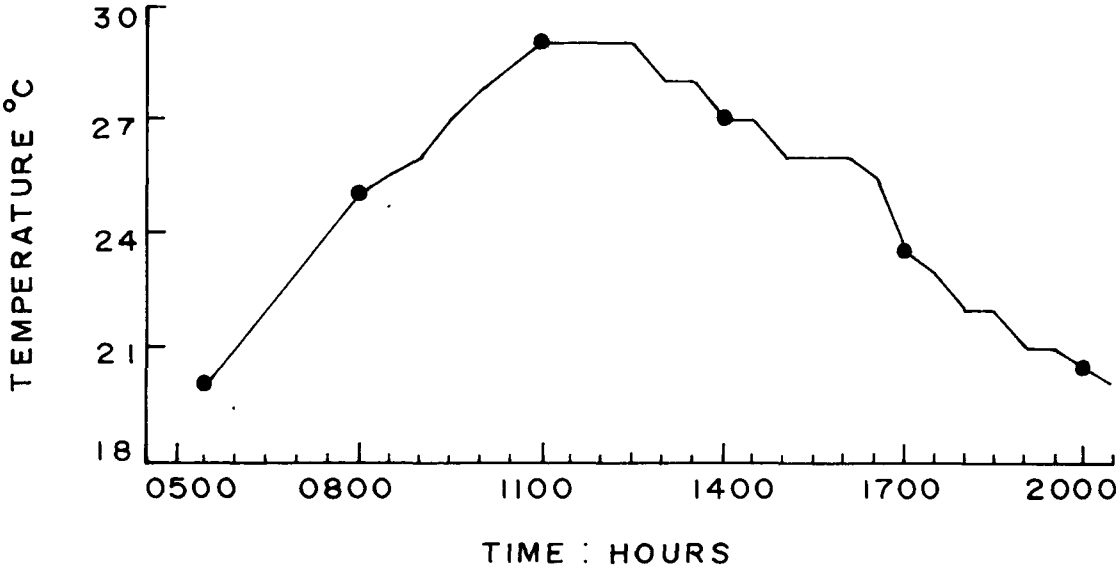


FIG. 4.19 CHANGES IN FIELD TEMPERATURE AT THE STUDY SITE. (SMIT). FROM 0500 HR TO 2000 HR.

Bufo melanostictus : On several occassions during field trips a large number of tadpoles of the common toad, *Bufo melanostictus* were seen in dense aggregations in ponds during the day time. Therefore observations on the daily cycles of distribution and activity were noted for the sake of comparison. It was seen that the tadpoles of this species started scattering in all directions in the evening time, while during the early hours of the day they started moving towards the edges of the pond. The tadpoles then densely aggregated and remained so through the hotter periods of the day. (Plates 4.3 A & 4.3 B). In the evening the tadpoles once again started moving towards the bottom of the ponds, Careful observations indicated that light triggered the activity of the tadpoles in the early mornings and once active they moved towards the shallower edges being attracted by heat and light. However the dense aggregations were the result of temperature gradients existing in the pond.

A

B

PLATE 4.3 A & B - DENSE AGGREGATIONS OF TADPOLES OF COMMON TOAD *Bufo melanostictus* DURING THE HOTTER PERIODS OF THE DAY.

DISCUSSION

5.1.1 FOOD PREFERENCE OF THE TADPOLES

The tadpoles of different species of anurans consume different food items is evident from following studies : 1) Jenseen (1967) while studying tadpoles of *Rana clamitans* reported that tadpoles fed on algae without showing any apparent food preference. However Sin and Gavrila (1977) reported that tadpoles of *Rana ridibunda* mainly consume algae, aquatic macrophytes and animal matter. Das (1979) found that tadpoles of *Rana hexadactyla* to feed mainly on aquatic vegetation. 2) Altig and Kelly (1974) suggested that species that might never feed on the bottom, might also contain some inorganic materials because of filteration of suspended particles. 3) Wilbur (1977) and Dash and Hota (1980) found tadpoles of *Rana tigrina* to be omnivorous, while Altig et al. (1975) and Sahu (1981) described the tadpoles of the species they studied to be omnivorous detritvores. The gut contents of the tadpoles of *Rana limnocharis* at stage 27 (hind limbs well developed) consisted mainly of detritus, vascular plant materials, mud and sand and non algal forms. The algal forms included Chlorophyceae, Bacillariophyceae and Cyanophyceae while the non algal forms included Ostracods, Rotifers and Cladocerns. The tadpoles of *Rana limnocharis* were thus found to be omnivorous and detritivores.

The guts examined at every stage were always found to be full with ingested matter, suggesting that they are continuous feeders. Continuous feeding habits has been reported for almost all tadpoles studied (Jenseen, 1967; Sahu, 1981). Sahu (1981) reported that, even during the severe winter months of December and January, the feeding activity of the tadpoles of *Rana alticola* did not decline. Thus feeding in general is a continuous activity.

The analysis of various groups of food items in the foregut and hindgut indicates the tadpoles of *Rana limnocharis* consumed highest percentage of Chlorophyceae which was 60.8% and 45.3% respectively. The percentage of other groups such as Bacillariophyceae was 18.2% and 16.3%, Cyanophyceae 6.4% and 24.6% and non-algal forms 14.6% and 13.8% respectively. Chlorophyceae was found to be most abundant in the habitat which was 42.1 % as compared to other groups which were less than 35%; as also reported by Sahu (1981). The percentage composition of various food items present in the gut was in correlation with the food items present in the periphyton. This reflects that food of tadpoles is dependent on the food items available in the system. As early as in 1928 Farlowe also arrived at similar conclusions from her study. She wrote that the gut contents of tadpoles gave a clear idea of the food organisms present in the system.

From all the foregoing studies it cannot be said whether tadpoles show any preference for food. They seem to be opportunistic feeders depending on whatever is available in the system.

DIGESTIBILITY

The examination of the percentage of food items from different parts of the gut of *Rana limnocharis* tadpoles revealed that most (about 80%) of them were in undamaged condition even in the hind gut indicating a poor digestive ability. Many food items in the hind gut were however hollow without their inner softer parts. This suggests that digestion may occur chemically rather than by a mechanical process as also pointed out by Wassersug (1975).

Certain authors such as Sahu (1981) suggested that the keratinized mouth parts play an important role in rupturing the cellulose walls of the vegetative food materials such as Chlorophyceae, and Cyanophyceae, while intestinal peristalsis serves as a mechanism for opening the algal cells. Wassersug (1975) argued that the keratinized mouth parts were important only in producing an initial suspension. They did not help in rupturing of the cellulose walls. Also due to the poor distribution of muscles in the intestinal walls, peristalsis cannot occur in tadpoles. He also suggested that when food is not abundant in the environment, the food consumed may stay in the

gut for a longer period of time and may be processed more thoroughly than when it is more abundantly available. It is difficult to comment on this aspect as we did not perform any experiment on this aspect.

5.1.2 VARIATIONS AT DIFFERENT TADPOLE STAGES

Little information is available on the changes in feeding habits of tadpoles at different larval stages. Altig and Dearman (1975) reported for 5 anuran tadpoles that they may shift their feeding emphasis in relation to the abundance of a given food item in their habitat i.e. the tadpoles consumed more of the vegetation which was abundant at every particular time, as also suggested by Jenseen (1967). Sabnis and Kolhatkar (1977) and Sabnis and Kuthe (1978) reported variations in the diet by components at different growth stages. Similar observations have been made by Jenseen (1967) and Christian (1982).The analysis of quality and percentage composition of the gut contents in relation to mouth parts at different developmental stages of *Rana limnocharis* tadpoles suggests that there exists some relationship in the food types, size and mouth gap and mouth parts at different stages (illustrated in Table 4.1).

So far as development of the mouth parts is concerned, at stages 21 to 24 the tadpoles depended mainly on food materials stored in the gut in all these stages. There was no feeding activity. The mouth parts were not well developed till stage 24.

The gut contents of the tadpoles at stages 25 to 28 included detritus, vascular plant material and some non-algal forms such as Rotifers, Ostracods and Cladocerans. The size of the mouth gap at these stages was 2-4 mm and the mouth parts were well developed at stages 26-28 with a dental formula of 1 : 1 +1/3.

At stage 29, the feeding activity was found to have slowed down as there were very few items in the gut at this stage. Few teeth were shed at this stage. At stages 30 and 31 there was no feeding activity and no food items in the gut. All the teeth were shed at this stage. The overall study suggests that size of food items consumed depended on the size of the mouth gap.

The metamorphosed juveniles of stage 32 assumed a carnivorous feeding habit. The presence of Formicoidea in highest percentage (65%) among various food items was followed by Oligochates, Coleopterans, Insect larvae, Arachnids and other food items which constituted the remaining 35%, substantiate the shift from herbivory at larval stages to carnivory at these stages.

Sahu (1981) also reported that in the tadpoles of *Bufo melanostictus, Philatus sp.* and *Rana alticola,* there was a considerable reduction is feeding activity during the later stages of larval development and also a shift from herbivory to carnivory during metamorphosis from larvae to juvenile stages. Jenseen (1967) found no change in the diet of *Rana clamitans till the*

emergence of fore limbs. However from this stage onwards they did not feed. The juveniles with a tail length of 7.00 mm started consuming on adult diet. He also showed that tadpoles consumed food items in relative propotions as they existed in water. Sahu (1981) found that at stage 42 (fore limb bud stage) the tadpoles of *Bufo melanostictus* and *Philatus sp* stopped feeding.

5.1.3 ARTIFICIAL FOOD

QUALITATIVE ANALYSIS

For laboratory culture of tadpoles different workers have used different food items. Gromko et.al (1973) and semlitsch and Caldwell (1982) used lettuce, John and Fenster (1975) used Purina Rabbit Chow, Wilbur (1977) used spinach leaves for culture of tadpoles in the laboratory.

Sabnis and Kuthe (1978) found the tadpoles fed exclusively on spinach and spirogyra showed maximum increase in their weight. However Mohanty-Hejmadi (1974b) found that a combination of Amaranthus leaves and boiled egg yolk promoted optimum growth, although this shortens the life cycle. Shivpal and Niazi (1979) used boiled spinach as food for successful rearing of *Bufo andersoni* tadpoles. Dash and Hota (1980) and Mishra and Dash (1984) used a mixed diet consisting of boiled Amaranthus species, boiled egg yolk, and cooked

goat meat for laboratory culture of anuran larvae.

In the present investigation the effect of different food items such as boiled egg yolk, cooked goat meat, ripe banana and mixed diet has been examined. Tadpoles fed either with cabbage or lettuce grow at a faster rate, attain a larger mean weight(0.230 g and 0.228g respectively) and metamorphose earlier (36 to 38 days) than those fed with other diets which grow at a slower rate, attain a lower body weight (0.100g-0.220g) and metamorphose by 39 to 56 days. The experiment indicates that either boiled cabbage or boiled lettuce can be used for successful culture of tadpoles within the laboratory.

Sabnis and Kolhatkar (1978) found tadpoles of *Rana cyanophlyctis* to successfully metamorphose when fed with spirogryra, as compared to those fed with Anaboena and Hydrilla. Dash and Hota (1980) on the other hand found that leafy vegetable diets alone produced deformities and diseases in *Rana tigrina* tadpoles. In the present experiment it was seen that leafy diet alone was sufficient for successful rearing of tadpoles. Apparently no deformities were seen in *Rana limnocharis* tadpoles. It was observed that small amounts of proteinaceous food such as boiled egg yolk can be added during the later stages of development .

Sabnis and Kolhatkar (1978) observed a decrease in body weight in the final stages of metamorphosis of *Rana cyanophlyctis* ie. after shedding of the tail. But sabnis and Kuthe (1978) did not observe such a decrease in body weight of *Bufo melanostictus* larvae.

In the present experiment a decrease in body weight from metamorphic climax stage was observed untill the completion of metamorphosis, while newly metamorphosed juveniles showed an increase in mean body weight.

QUANTITATIVE ANALYSIS

Certain workers such as Mohanty - Hejmadi (1974), Wilbur (1977) and Sabnis and Kuthe (1978) have studied the effect of different food types on the growth of tadpoles but only a few have studied the amount of food to be used for rearing of tadpoles under laboratory conditions. Savage (1952) reported that food is a limiting factor for the tadpoles under natural conditions. Wilbur (1977) and Dash and Hota (1980) reported on the interactions of food level and larval density.

In the present study it was observed that tadpoles reared with a ration of 0.1g and 0.2g of boiled cabbage attained a mean weight of 0.140g and 0.150g respectively and metamorphosed by 41-42 days. The tadpoles reared with 0.6g attained a mean weight of 0.230g and metamorphosed by 36-37days, where as tadpoles reared at 0.8g and 1.0g food level, attained mean weights of 0.228g and 0.220 and metamorphosed by 37 to 38 days. The results indicate that 0.6g food level was optimum.

The growth rate increases (Fig. 4.1) as the food level increases only to a certain level. If the food level is too high

also, growth rate is reduced. In the present experiment, the slow growth at high food level of 1.0g may be due to the putrefication of unutilized food materials, leading to a depletion of oxygen in the medium. However the time of metamorphosis was not significantly affected by food level.

Tadpoles with 0.6g food level reached the metamorphic climax by 27 days, where as these with 0.1g food level reached metamorphic climax by 33 days (see table 4.14). Travis (1984) found that low food levels resulted is decreased average larval growth, size at metamorphosis and lengthened the larval period in *Hyla gratiosa*. In the present study it was observed that low food level of 0.1g/5tadpoles/2days resulted in a slower growth rate and lower body weight at metamorphic climax, where as high food level of 1.0g/5tadpoles/2days resulted in a slightely lower body weight in comparision to 0.6g food level. However the time required for completion of metamorphosis was not significantly affected by food level.

5.2.1 DENSITY DEPENDENT GROWTH AND METAMORPHOSIS OF TADPOLES

The variatiations in growth rates observed in density experiment have been analysed recently by Wilbur (1977) and Dash and Hota (1980). According to them a greater proportion of variance observed in growth rate can be accounted for by initial density of the

population, while within group variations are due to mortality effects. The remaining variations can be explained by chance physiological or genetic differences among individuals. In the present experiment the eggs of *Rana limnocharis* were collected from a temporary breeding site near Shilling. The tadpoles hatched from a single spawn were used for each experiment to reduce environmental or allelic heterogeneity among individuals. The variations in growth rate and metamorphosis observed in the laboratory experiments may be due to the initial density of the population, while within group variations may be the result of mortality effects.

LARVAL GROWTH AND BODY WEIGHT

Most of the earlier workers have indicated that growth and differentation are retarded at high densities (eg. Licht, 1967; Wilbur, 1972,76.77; Gromko et.al. 1973; Wilbur & Collins, 1973; John & Fenster, 1975; Smith Gill & Gill, 1978). This has further been confirmed by workers such as Dash & Hota (1980), Semlitsch and Caldwell (1982) and sokol (1984).

In the present study it was seen that the tadpoles of *Rana limnocharis* reared at low densities such as 4, 16, 32 tadpoles/ tray attain high body weights of 0.228, 0.230g and 0.217g respectively at metamorphic climax. At high densities of 128 and 256 tadpoles, they attain body weights of 0.140g and 0.124g respectively at metamorphic climax. However, in high density populations there

was a dichotomy seen in the response of growth rate of individual tadpoles. Some tadpoles that gained an early growth advantage presumably metamorphose at the minimum size, possibly in order to escape the density stress. The remaining smaller growing tadpoles, if they survive may then be able to grow as the effective density is lowed and more food is available to them. Thus in the present experiment a minimum body weight ie. threshold size of 0.14g was observed for the tadpoles to metamorphose. A number of other workers such as Dash & Hota (1980), Wilbur (1977), Semlitsch & Caldwell (1982) & Sokol (1984) have reported on the threshold size of different anuran species. Wilbur and Collins (1973) proposed in their growth-based model of metamorphosis that amphibian larvae must attain minimum threshold size before metamorphosis can occur. Whether a tadpole metamorphoses at the threshold size or continues to grow depends on its recent growth history : Slow growing tadpoles metamorphose at, or near the lower threshold, while fast-growing tadpoles continue to grow and transform at a larger size. However it is well established that amphibian larvae growing slowly due to low temperature regimes metamorphose at a larger size than do larvae reared at higher temperatures (see Smith-Gill and Berven, 1979 among others), hence a generalized positive physiological relationship between growth rate and body size at metamorphosis does not exist.

GROWTH RATE, METAMORPHOSIS AND SURVIVORSHIP

According to Semlitsch and Caldwell (1982) the survivorship of *Scaphiopus hoolbrookii* tadpoles to metamorphosis was a negative density - dependent function. Wilbur (1977) and Dash and Hota (1980) reported the same for *B.americanus* and *R.tigrina* tadpoles respectively. According to Wilbur (1977) at high densities, few individuals grow at the expense of the smaller members of the chorot. The results of the present experiment also support the view of Semlitsch & Caldwell (1982), as it was observed that there was more mortality and fewer individuals metamorphosed at high densities as compared to those at low densities.

The number of days to metamorphosis for *Rana limnocharis* tadpoles was directly related to initial density. The larvae grown at low densities metamorphosed by 36 to 40 days, where as larvae reared at high densities metamorphosed by 46 to 50 days (see Fig. 4.1). Similar results have been reported by semlitsch and Caldwell (1982) for *Scaphiopus holbrookii* tadpoles; by Dash and Hota (1980) for *Rana tigrina* tadpoles and by Sokol (1984) for *Litoria ewingi*. Smith - Gill and Berven (1979) however found no significant correlation between growth rate and length of larval period of *Rana pipiens* reared at a constant 18°C, or for *Rana sylvatica* reared at room temperature. Based on their work

they proposed the differentation - rate model for prediction of metamorphosis. In contrast to this Wilbur & Collins (1973) have proposed, the threshold-size model for predicting metamorphosis.

CAUSES OF RETARDATION IN GROWTH.

Several explanations have been put forward to explain the slow growth rates of the tadpoles in high densities.

1) According to Richards (1958) and other workers such as Sokol (1984), slow growth rate of tadpoles in high density populations may be due to an interference mechanism of competition in which large growing tadpoles produce a substance that inhibits the growth of smaller members of the population.

2) Gromko et.al (1973) and John and Fenster (1975) have argued that behavioral interactions and their influence on the hormonal control of growth can account for the slow growth rates in high density populations.

3) In both the previous studies food was not a limiting factor. However some workers such as Dash and Hota (1980), Wilbur (1977)& Brockelman (1969) studied this effect by using limited amount of food. They have explained the slow growth rate due to competition for food, a situation more akin

to the natural occurance of crowding (Southwick, 1976).

In the present study both the food and space were kept constant. Slow growth rate in high density populations observed in the present experiment may be due to the individuals competing for the limited amount of food. As the initial density is increased more individuals have to compete for the same amount of food. In this process, some individuals which gain an early growth advantage consume more food, thus growing to a larger size and metamorphosing earlier than the smaller ones which have a lowered probability of surviving till the completion of metamorphosis. It was also observed that when fresh food was added to the trays, the larger growing tadpoles consumed more of the food and prevented the smaller tadpoles from consuming food. Savage (1952) also reported similar observations under natural conditions for many tadpole species.

5.2.2 CULTURE MEDIUM AND DEVELOPMENT

There are a very few reports on this aspect. Singh et al. (1980) found that aeration causes efficient utilization of food by fish fingerlings while Wassersug and Feder (1983) found that aquatic hypoxia reduced locomotar stamina (time of fatigue) in anuran larvae most conspicuously in *Bufo americanus* larvae. Noble (1931) on the other hand attributed the cause of slower development and differentiation among the tadpoles inhabiting oxygen deficient water to a decrease in thyroid size.

In the present study the effect of culture medium on development has been investigated. It was seen that the group in which water was regularly changed, the tadpoles attained a mean weight of 0.230 g and metamorphosed by 36 to 37 days, while the group in which the culture medium was not changed, the tadpoles attained a mean weight of 0.156 g and metamorphosed by 50 to 52 days. The slower growth of the tadpoles in the 2nd group may be due to the fouling of the medium and also due to deficiency of oxygen.

5.3.1 TEMPERATURE AND DEVELOPMENT

The effect of temperature on development is a selective factor acting upon life history (Berven et al. 1979), mating systems (Howard, 1978) and habitat selection in amphibians. Low temperatures causing slower development (Moore, 1939), may limit where a species can breed if temporary aquatic habitats dry before metamorphosis can occur or if prolonged exposure to predators reduces the number of eggs hatching or larvae metamorphosing. High temperature may result in developmental abnormalities which likewise reduces hatching success (Howard, 1978).

The developmental response to temperature variations has been studied in a number of amphibians (see Townsend & Stewart, 1986). Environmental temperature is a major proximal factor in the growth of anuran tadpoles. The differential sensitivity of growth to temperatures has been demonstrated for many organisms (Etkin, 1964). At low temperatures the maintainance of an ectotherm is less and

the organism can channel a greater proportion of its energy into growth. It is also well known that thyroxine promotes differentiation, while inhibiting growth (Etkin, 1964). and low temperatures inhibit thyroxine output (Etkin, 1964) and at the same time render amphibian tissues insensitive to thyroxine (see Fry 1972 among others). Thus either direct temperature effects the developing tissue or indirect temperature effects mediated by thyroxine are sufficient to explain temperature dependent growth.

In the present study the effect of high and low temperatures on the development of tadpoles was examined. The tadpoles reared at high temperature of 32°C, attained a mean weight of 0.185 g and metamorphosed by 34 to 36 days. At low temperature of 10°C, the development proceeded slowly and the tadpoles attained a mean weight of 0.280 g, but did not metamorphose till 56 days. At room temperature ($\bar{x}$ 23°C) the tadpoles attained a mean weight of 0.230 g and metamorphosed by 36-37 days. Thus it was seen that at high temperatures the tadpoles attained a smaller body weight and metamorphosed slightly earlier than those reared at room temperature. At low temperatures they attained a larger body weight and did not metamorphosed till 56 days.

The subtropical climate of Shillong provides an average minimum temperature of 17°C and an average maximum of 24.3°C during the summer months. The range of normal development of tadpoles observed in the laboratory is well within the range of temperatures experienced in the field

5.2.2 THERMAL TOLERANCE LEVELS (CTM_{ax} AND CTM_{in}) AND THERMAL ACCLIMATION

a) Thermal tolerance plays a vital role in the normal activity and distribution of amphibians (Aurelia Pawloska - Indyk, 1978). The habitats of tadpoles are generally exposed to a wide range of temperature fluctuations. In order to over come this difficulty the tadpoles appear to have developed a high range of thermal tolerance. (Heatwole, et al., 1968).

Heatwole et al.(1968) and Krakauer (1970) have given CTMax values from 41.5°C to 42.5°C for *Bufo marinus* tadpoles. Sherman (1980) reported a CTMax value of 42.5°C for the tadpoles of *Bufo woodhousii*. In fact, the highest CTMax ever reported for a tadpole is 43.3°C in *Gastrophryne carolinensis* (Cupp, 1974).

Very little work has been done on the critical thermal minima (CTMin) of anurans. Krakaur (1970) reported 9°C as CTMin for *Bufo marinus* but did not mention the stage of development. Floyd (1983) determined the CTMin for *Bufo marinus* at various stages of development.

In the present study the CTMax of the tadpoles of *Rana limnocharis* at stages 26-27 (hind limbs developed) was found to be 41.5°C while the CTMin at these stages was found to be 5.8°C. The high thermal tolerance level of *Rana Limnocharis* tadpoles can be discussed in terms of their adaptation to breeding in temporary habitats. Heatwole et al (1968) suggested that tadpoles

in temporary ponds or shallow pools may be subjected to wide fluctuations in temperature and may be vulnerable to increase in water temperature beyond their tolerance limits. Floyd (1983) also correlated the high temperature tolerances of *Bufo marinus* with the breeding habitats. The high thermal tolerance level of *Rana limnocharis* seems to be of a selective advantage for them in shallow temporary pools. Cold tolerance would not be so important to the tadpoles as the water bodies often remain warmer than the air over night. Thermal adaptation however involves more than thermal tolerance limit, though high tolerance limit may be indicative of a general system of responses adjusted to high temperatures. In case of tadpoles living in temporary ponds, maximization of rate growth and development would increase the probability of completing metamorphosis before drying up of ponds. In so much as developmental rate is thermally dependent, maintainance of high body temperature would contribute to quicker metamorphosis and one would expect thermal responses to be adjusted towards using high environmental temperatures.

b) Thermal acclimation

The effect of thermal acclimation on thermal tolerance is well known in adult anurans (Brattstrom & Lawrence, 1962; Heatwole et al., 1968; Dunlop, 1969; Hutchison & Ferrance, 1970; Claussen, 1973), but only a few studies have utilized larvae (Brown, 1969; Dunson, 1977; Cupp, 1980).

Thermal acclimation, which is often used as a measure of other metabolic changes in an animal, is usually measured as a change in the CTMax or CTMin or as a change in the ability to survive at higher (or lower) temperatures for longer periods. A number of workers have studied this aspect. Temperature tolerance of adult *Bufo marinus* has been determined using a variety of methods and end points and have resulted in CTMax values from 30.5°C to 42.0°C depending on the acclimation temperatures which ranged from 7°C to 38°C (See Floyd, 1983). Brattstrom and Lawrence (1962) reported the CTMax of adult *Rana clamitans* to be about 35°C at 23°C acclimation, while Willhite and Cupp (1982) reported a CTMax range of 39.0°C to 40.4°C at acclimation temperatures ranging from 10°C to 30°C, for the same species. Claussen (1973) found that the CTMax of *Hyla regilla* was significantly above that of *Ascaphus truei* at acclimation temperatures of 0°C, 10°C and 20°C. This may be due to the fact that *Hyla regilla* is eurytopic species found in a wide variety of thermally diverse habitats, while *Ascaphus truei* a highly stenotopic anuran found only in cold mountain stream habitats. Cupp (1980) found significant differences in CTMax between 20°C and 30°C than between 10°C and 20°C in each of the species he examined. This indicated an increased capacity for acclimation to higher temperature. However Hutchison (1961) found no significant difference in CTMax on acclimation at low temperatures. Also Miller and Packard (1977) found no influence of 3-4 weeks acclimation at 5°C and 2°C of

Pseudacris triseriata.Dunlop (1968) observed that increasing the temperature of acclimation by 10°C in the range from 14 to 35°C will result in a corresponding increase in CTMax from 0.6°C to 2.1°C in *Pseudacris triseriata* and *Acris creptians*.

In the present study, the CTMax of tadpoles of *Rana limnocharis* at stages 26-27 were determined at different acclimation temperatures. The tadpoles acclimated at 10°C had a mean CTMax of 37.2°C while those acclimated at 23°C had a mean CTMax of 41.5°C. The tadpoles acclimated at 35°C had a mean CTMax of 42.1°C.

The data on acclimation in this species suggests that there is in fact some ability to physiologically adjust within limits. The ability to adjust their CTMax and presumably other aspects of metabolism (Fry, 1972, 1958; Dawson & Bartholomew, 1956) allows them to survive short or longer periods of adverse temperatures which, if behavioral responses failed be lethal.

Thus acclimation is one mechanism that allows organisms to exploit or survive in the extremes of environmental conditions within their habitat. It is important for survival, for efficiency and for toleration of the extremes of the environment. This extensions of the physiology of the organism further allows for occupation and adaptation to new environments, hence in the formation of new forms.

5.3.3 THERMAL TOLERANCE AND DISTRIBUTION

In an earlier study, Roy and Khare (1979) determined the embryonic limiting temperatures of *Rana limnocharis* and found a temperature tolerance range of 5°C to 28°C. The high thermal tolerance (CTMax) of 41.5°C and a low tolerance level (CTMin) of 9.6°C of the tadpoles determined in the present study, indicates that it has a wide range of thermal tolerance and can adapt itself to different habitats, a fact that can be proved by its wide spread distribution throughout the Eastern tropics both in cold climates at high altitudes and in the hotter plains. This suggests that this species may have different thermal groups i.e. cold-adapted and warm adapted. Kuramoto (1975) has also studied this aspect in relation to 12 species of frogs and reported that species which breed in summer or in warm water are smaller than those which breed in winter or in cold water.

5.3.4 THERMAL TOLERANCE : CTMAX

EMBRYONIC STAGES

The pattern of changes in CTMax for various anurans has been investigated by a number of workers. According to certain workers such as Kuramoto (1978) critical temperatures for short exposures are higher than those for standard exposure from stage 3 to 20. Kobayashi (1962) determined the maximum temperature limit for *Rana japonica* embryos to be about 28.5°C, while Herreid

and Kinney (1967) exposed embryos of *Rana sylvatica* at various developmental stages to high temperatures for 1 hr. Their data shows that the critical temperature for the most susceptible stage-3 embryos is about 28°C. Brown (1967 a,b) determined the critical temperature for *scaphiopus hammondii* embryos to be 30-32.5°C, where as stage 3-5 embryos can tolerate 36.5°C during a 3 hr exposure. similar pattern of tolerance has been reported by Zweifel (1977) for *Rana sp (pipiens group), scaphiopus bombifrons, S. Couchii* and *B. cognatus*.

In the present study on the embryos of *Rana limnocharis* exposed to high temperatures for 2 hr it has been found that there is a sharp increase in CTMax from 37.6°C at stage 4 (four cell stage) to 41.8°C at stage 11 (blastopore stage) and then remains fairly constant till stage 17 (tail bud stage) at 41.8°C. This is followed by a decrease in CTMax to 41.1°C at stage 22 (hatching stage).

Kuramoto (1978) while studying critical temperatures of six anuran species noted that the tolerance level of these anurans were low in initial stages of development, but increased with development towards a plateau from gastula to late embryonic stages and then decreased in early larval stages. Also a sharp rise in tolerance at the gastrula stage has been noted by Brown (1967). It remains so till the late embryonic stages and then declines rapidly at stage 25. Herreid and Kinney (1967) made

similar observations for *Rana sylvatica* embryos at stage 25. This pattern of changes in tolerance level may be true for the species studied by other workers such as Brown (1967) and Zweifel (1977).

Because the tolerance level changes with the stages of development, the standard critical temperature is determined at stage 3 which is the most susceptible stage (see Kuramoto, 1978 among others). Embryos that are able to survaive this stage have greater chances of surviving to later developmental stages.

In the present experiments the embryos of *Rana limnocharis* at stage 4 (four cell stage) exposed to high temperatures for 2hr had a tolerance level of 37.6^{o}C. The embryos exposed to longer periods to high temperature at this stage as well as other stages, resulted in fewer embryos surviving and normally developing.

TADPOLE AND JUVENILE STAGES

The upper limit of critical temperature tolerance (CTMax) of amphibian tadpoles has been reported to change with developmental stages by workers such as Hathaway (1928) for *Bufo americanus*, Herreid and Kinney (1967) for *Rana sylvatica*, Heatwole et.al.(1968) for *Leptodactylus albilabris* and *Bufo marinus*, Cupp (1980) for five salientian species, Sherman (1980) for *Bufo woodhousii* and

Floyd (1983) for *Bufo marinus*. They found that the CTMax increases gradually during the late larval stages and decreases rapidly at metamorphic climax and then again gradually increases in postmetamorphic animals. Drupe and Petranka (1985) also observed that the mean temperature preference increased with developmental stages of the larvae and peaked at or shortly before metamorphic climax. Cupp (1980) suggested that this may be a wide spread phenomenan for salientaian species from the temperate regions.

In the present study slight increase was noted in the CTMax of larval stages of *Rana limnocharis* from 41.4^{o}C at stages 22-25 to 41.5^{o}C at stages 26-27. It decreased to 40.6^{o}C when the limbs developed at stages 28-29 and to 39.5^{o}C at stages 30-31. Newly metamorphosed froglets of stage 32 had the lowest CTMax of 37^{o}C. Post metamorphic juvenils again showed an increase in their CTMax's as the juvenils of S-V length 28-30 mm had a CTMax of 40.5^{o}C. On several occasions early as late stages were seen is very shallow patches of water (see plates 4.2 A,B,C,D). Even though the late stages had a lower CTMax it was well above the temperatures experienced in the field

So far as the decrease in CTMax at metamorphic climax is concerned, following views have been put forward.

i) Hoppe (1978) studied the changes in tolerance levels during development of *Pseudacris triseriata* and suggested that

the CTMax of adult frogs simply reflects adaptations of tadpoles to high environmental temperatures and that there is no terrestrial selection to high thermal tolerance.

ii) Cupp (1974,80), on the basis of observations of 5 salientian species suggested that the decrease in CTMax at metamorphic climax was a manifestation of the adjustment downward in the thermal regime of the species to be able to tolerate the low temperatures of winter. However he had difficulty in explaining why adult toads had higher CTMax than metamorphosing individuals.

iii) Sherman (1980) argued that since metamorphosis was a period of great biochmical and morphological rearrangements (Etkin, 1964) the animal being under stress at this time was merely unable to tolerate high temperature rather than undergoing some adaptive shift in tolerance. The gradual increase in CTMax from post-metamorphic to adult toads noted suggests that the physiological systems underlying thermal tolerance change or mature following metamorphosis untill the adult condition is reached. Thus rather than being an adaptation to the decreased temperatures to which metamorphosed anurans are exposed, the drop in CTMax during transformation may simply be an indication to the stressful nature of metamorphosis which would result from intensive differentation occuring during this process. This is supported

by the observation that metamorphosing animals required upto 4 hr to recover from a CTMax determination, while animals at other stages recovered in less than a minute.

iv) Kollros (1981),however is of the view that the ontogenetic shifts in thermal sensitivity may be due to a turn over or maturation of neurons during development. This view has been supported by Drupe et.al. (1982).

v) White and Nicoll (1981) have suggested that these changes correlate with the changes in plasma levels of hormones such as thyroid, prolactin and adernal corticosteroids during metamorphosis.

The metamorphic climax in *Rana limnocharis* is also a phase of several drastic changes in its morphology as well as physiology. Its tail is completely resorbed and the limbs take over the function of locomotion; there is complete shedding of larval teeth and changes in the shape and size of the mouth well adapted for carnivorous diet; and new enzyme systems appear and regulate the physiology and metabolism suited for adult amphibious life. This period of reorganisation is of great importance during the life cycle as it enables the tadpoles t_O aquire a terrestrial life. The behavioural thermoregulation seems to play an important role at this stage. The fact that newly metamorphosed froglets of *Rana limnocharis* show a lower CTMax($37^{o}C$) also suggests that

it has just passed through a period of stress due to various biochemical and metabolic changes in their physiology as reported by Sherman (1980).

Sherman (1980) observed that "adult parts" (Limbs) of metamorphosing tadpoles stopped moving at lower temperatures than "tadpole parts" (tails). He correlated the changes in CTMax to this behaviour. The present observations also reveal that in metamorphosing tadpoles, the limbs stopped moving at temperatures slightly below the temperatures at which the tails stopped moving.

5.3.5. THERMAL TOLERANCE : CTMIN

TADPOLE STAGES :

Little work has been done on the changes in CTMin during development of anurans. Floyd (1983) reported that CTMin changed more dramatically with stage of development than did the CTMax. The CTMin curve had no platau like the CTMax curve but did indicate a sharp reduction in tolerance to low temperatures (increase in CTMin)from stage 42 to 44. He stated that it could be due to the fact that larval parts are more tolerant than the adults. In the present study the CTMin was found to decrease from 7.6^{o}C to 5.9^{o}C from early larval stages to stages with hind limbs developed. This was followed by a sharp increase in CTMin to 10^{o}C at newly metamorphosed froglet stages. However older juveniles showed a slightly lower CTMin. Floyd (1983) did not observe any

decrease in CTMin during the late stages of development. However in the present study older juveniles showed a lower CTMin than the newly metamorphosed juveniles. There are no more reports on the changes in CTMin to make a comparative account. The changes in CTMin at tadpole stages are not so important as they are usually present during the summer months when environmental temperatures are high.

ECOLOGICAL SIGNIFICANCE OF THERMAL TOLERANCE LEVELS

EMBRYONIC STAGES :

The ecological significance of the variation in embryonic temperature tolerance of various species such as *S.hammondii, Rana aurora, R.pretiosa,* has been discussed by a number of authors (Ballinger and Mc Kinney, 1966; Brown, 67 a,b Brown 1969, , 1975, Licht, 1967, Moore, 1939; Volpe, 1953, 1957; Zweifel, 1968). Kuramoto (1978) reported that the species which breed at higher environmental temperatures, show a higher level of thermal tolerance, while species which breed at lower environmental temperature have lower levels of thermal tolerance. Zweifel (1977) reported that the upper limit of 2 hr temperature tolerance reached by any anuran is about 41°C, but Kuramoto (1978) determined the value for *Rana limnocharis* to be 43°C, the highest value reported for anurans so far.

In the present study the upper limit of 2 hr temperature

tolerance for *Rana limnocharis* embryos determined is 41.8°C. This value is lower than that reported by Kuramoto (1978), This difference may be because it is a sub-tropical population of this species.

As a number of workers such as Brown 1967a, Herreid and Kinney,(1967) and Zweifel, (1968,77), have discussed that the high temperature tolerance level of the embryos of *Rana limnocharis* can be related with the environmental temperatures at the breeding site. The habitats are usually small, shallow ponds or pools which may experience high temperatures during the day time. During the course of adaptive divergence, critical thermal maxima for embryonic development may have been modified by selection pressures directly relating to the environmental temperature. Kuramoto (1978) in his study observed that all the species lay eggs in open still waters at night time when the water temperatures are lower than in day time, and hence the most sensitive stages for embryonic development pass through conditions which do not reach the upper critical thermal limit. However sometimes the embryos may be exposed to high temperatures which may be lethal. Thus to have high thermal tolerance is an adaptive feature as far as breeding ecologies are concerned.

Early larval stages on the other hand show a slightly lesser thermal tolerance. This may be due to the fact that behavioral thermoregulation seems to compensate for the lower tolerance of the tadpoles.

Not much information is available on the mechanisms of

of embryonic thermal tolerance. According to some authors the levels of temperature tolerance of hybrid embryos are not intermediate but like their maternal parent. Volpe (1957) reported that the hybrid embryos from the cross between *Bufo americanus* and *Bufo woodhouseii* were maternal in their temperature tolerance, while Brown (1967) showed that hybrid embryos between species of *Scaphiopus* had the same level of temperature tolerance as the control embryos. Thus certain cytoplasmic factors may be responsible for determining thermal tolerance levels. A sharp rise in tolerance level during early cleavages may be a result of progressive partitioning of cytoplasm or if some biochemical processes are involved may be modified by maternal mRNA transcribed during the growth period of oogenesis.

Several workers such as Moore (1939, 42), Zweifel (1968), Bachman (1969), Mclaren and Cooley (1972), have described temperature adaptation to be one important characterstics of amphibians directly related to their geographic distribution, breeding habits and rates of development. Roy and Khare (1979) determined the limiting temperatures of *Rana limnocharis* to be between 5°C to 28°C. This wide range of adaptation and wide range of CTMax levels (5.8°C to 41.5°C) as seen in the present study may be related to their wide spread distribution.

LARVAL STAGES

The ranges of thermal tolerance as an adaptive character in

an organism is a result of different selection pressures in the environment. (Snyder and Weathers, 1975). The thermal regime of a species must encompass the highest and the lowest temperature experienced in the habitat. It would be disadvantageous for terrestrial stages to maintain the ability to tolerate higher temperatures than necessary. If there is no selection pressure to maintain high termal tolerance this ability would be lost. Also selection should favour mechanisms that accelerate development towards the end of the larval period, particularly during metamorphic climax when growth slows and larvae become increasingly susceptible to habitat deterioration (Wilbur, 1980) and predation (Arnold and Wassersug, 1978). Selection of warmer temperatures may be a manifestation of such selection.

According to Floyd (1983) it would be of adaptive advantage to the larvae of *Bufo marinus* to have greater tolerance to high temperatures than adults since this species often breeds in temporary pools which may become hotter than the shaded places of the adults during the day. Cold tolerance would not be so important to larvae since water bodies often remain warmer than the air overnight. The stages immediately prior to metamorphosis congregate in the very shallow water is likely to have the greatest range of temperatures of any of the habitats occupied by *Bufo marinus* at any stage in its life cycle.

5.3.6 DAILY RHYTHMS

Willhite and Cupp (1982) reported that the CTMax for the tadpoles of *Rana clamitans* increased sharply from about 39.4°C at early morning hours to about 40.2°C during the mid day and remained so through the hotter periods of the day. There was a sharp decrease in CTMax to 39.8°C towards the late evening hours

In the present investigation the CTMax for the tadpoles of *Rana limnocharis* was found to increase from 40.6°C during early morning till about afternoon to 41.6°C and then remained fairly constant through the hotter periodsof the day. This was followed by a decrease to 40.8°C at 2000 hr. Thus although the pattern is similar to Willhite and Cupp (1982), the CTMax at any time of the day was higher than that reported by them. This could be due to the fact the *Rana limnocharis* being a subtropical species has much higher CTMax than other species.

The diel changes in the CTMax can be explained by relating them to the daily changes in temperature of the shallow water bodies. The tadpoles have a lowest CTMax in the early mornings, when the environmental temperatures in the field are lowest. The CTMax increases in direct relation to environmental temperature, which increases from early morning to mid day. It was observed that there were significant differences (at 0.01 level) in most of the combinations except few such as 0800 hr-1100 hr and 1100 hr-1400hr. (see table

4.31. This indicates that the CTMax reaches a maximum and remains so throughout the mid day, ie the hotter periods of the day.

Similar rhythms though with low CTMax values have been reported for adults of *Litoria caerulea* by Johnson (1971,b) and for *Bufo marinus* by Johnson (1972). Mahoney and Hutchison (1969) found that daily variations in CTMax for *Rana pipiens* and *Hyla labialis* may be adaptive, while Dunlop (1969) also discussed that CTMax were adaptive in nature.

ECOLOGICAL SIGNIFICANCE

The presence of a daily rhythm of thermal tolerance in *Rana limnocheris* tadpoles reflects that when the environmental temperatures are low the CTMax is low and correspondingly high when environmental temperature is high. Since *Rana limnocharis* tadpoles are present in aquatic environment, they may be vulnerable to increase in water temperature beyond their tolerance limit, it would be a selective advantage for such tadpoles to have a high termal tolerance.

In addition to the survival value, higher thermal tolerance levels of the tadpoles would permit them to select regions in the ponds where temperature sometimes may reach very high level. The maintainance of high temperatures in turn increases the rate of growth and development of tadpoles and helping in the quicker completion of metamorphosis as also argued by Willhite and Cupp (1982).

Dunlop (1969) suggested that variations in heat resistance might be a product of some underlying physiological rhythm and that it might be adaptive. During the summer, breeding populations of *Bufo marinus* can often be found in ephemeral ponds during the day . A daily rhythm in heat resistance might have survival value by increasing resistance during the mornings and afternoons when the temperatures are high. In the present study the possession of a daily rhythm of CTMax appears to be an adaptation to breeding in temporary vernual water bodies which are exposed to great temperature fluctuations during the day time.

DAILY CYCLES OF DISTRIBUTION AND ACTIVITY UNDER NATURAL CONDITIONS

Workers such as Brattstrom (1986) , Tevis (1966) and Beiswenger (1975, 1977) have observed that tadpoles of *Bufo species* move into warm areas of the pond as they become thermally stratified ie a temperature gradient develops in the mornings. They spend the day time in the warmest regions of the ponds available to them.

As to how different species behave in relation to their distribution pattern and temperature variation distribution, it may be worthwhile to compare the observations on *Rana limnocharis* and *Bufo melanostictus* tadpoles.

Rana limnocharis tadpoles :-

Field observations on the daily cycles of activity of *Rana limnocharis* tadpoles indicated that the tadpoles more frequently inhabit the warmer areas of the habitat during the day time ie. the number of tadpoles seen towards the edges and the upper regions of the water bodies was more than those present at the lower or bottom regions. They also showed intense feeding activity during the hotter periods of the day. However no distinct aggregation formations were seen. This may be due to the fact that in most of these habitats the water depth was not much for distinct thermal gradients to develop.

Bufo melanostictus tadpoles

Observations on the activity cycles of tadpoles of *Bufo melanostictus* showed that these tadpoles formed dense aggregations during the day time. The tadpoles were seen scattered in all directions towards the evening time, while in early mornings they were seen near the edges of the ponds. By mid day they aggregated in dense formations and remained so through the hotter periods of the day. Though light seemed to trigger the movements of the tadpoles in the early mornings role of thermal gradients in the pond in this behaviour cannot be ruled out.

ADAPTIVE SIGNIFICANCE

The aggregative behaviour of the tadpoles of *Bufo melano-*

stictus observed in the present study as well as there reported by other workers such as Johnson (1972), Beiswenger (1975,77) can be analysed as follows. By following a thermal gradient to warm areas, the animals gain a higher body temperature and hence more rapid metabolism, which in turm results in a faster rate of development and shortens the duration of metamorphosis. This may culminate in early metamorphosis and survival of species in those areas where ponds dry up rapidly or where possibility of predation is high. However, Sahu (1981) has argued contrary to this view According to him temperature does not effect the timing of metamorphosis. More field studies are needed to verify this.

Brattstrom and Warren (1955) observed that in tadpole aggregations of *Hyla regilla* most of the tadpoles were oriented with their tails pointed to the sun. They were of the view that this would probably expose the greatest dorsal surface to the suns rays and that a group of tadpoles might be able to absorb more heat as a mass than as isolated individuals.

In the light of present knowledge, the aggregative behaviour seen in tadpoles under natural conditions is probably due to more than a single factor. They are controlled by a complex of several factors operating simultaneously or in a sequence in a system. These factors include sunlight, predators, parasites, food,water depth, oxygen tension and other limnological conditions. The

evolution of aggregative behaviour may have begun with relatively simple thermotactic and phototactic responses.

SUMMARY

The present study deals with food, density and temperature tolerance of the tadpoles of *Rana limnocharis*, Weigmann, in relation to its growth and metamorphosis.

FOOD ASPECTS

The investigations on food aspects include analysis of (1) Gut contents and (2) Artificial diet on the growth and metamorphosis of tadpoles.

(1) Gut contents

The gut contents of fully developed tadpoles (stage 27) have been analysed and a comparison has been provided for pre feeding and post feeding stages of the tadpoles. In the fore gut Chlorophyceae was 60.8%, Bacillariophyceae 18.2% Cyanophyceae 6.4% and non-algal forms 14.6%. Detritus and mud were present in abundant quantities. In the hind gut the percentage of these items. was Chlorophyceae 45.3%, Bacillaiophyceae 16.3% Cynophyceae 24.6% 24.6% and Non algae forms 13.8%. Detritus and mud were present in abundant quantities.

In the perphyton samples of the pond system from where the tadpoles were collected had chlorophyceae 42.1%, Bacillariophyceae 35.3%, Cyanophyceae 5.1% and Non-algae forms 17.5%.

During the stages 22 to 24 there was no feeding activity. The tadpoles depended for nourishment on stored yolk. The mouth is small and not well developed. These stages have been designated as prefeeding stages.

The stages 25 to 28 were found to be the active feeding stages. At stage 25 the gut contents included detritus, Vascular plant materials and some Non algal forms such as Diatoms and Rotifers. The mouth is slightly wide and contains one continous row and one interrupted row of teeth on the upper labial fringe while the lower labial fringe has 3 faint but distinct rows of teeth.

At stages 26 to 28, the gut contents included detritus, mud, vascular plant materials and larger food items such as Filamentous algae, Ostracods and other Zoephlanktons. The mouth parts completely developed with a dental formula of 1 : 1 + 1/3 at these stages. There was a size relationship between mouth gap and food items taken.

At stages 29 to 32 the tadpoles did not feed and they are designated as the post feeding stages. The mouth parts are shed by stage 30 and by stage 32 the mouth becomes transformed into adult type with a prominent tongue which is utilized for feeling on a carnivorous diet.

2) Artificial diet

The effect of following artificial food items was examined on the growth and metamorphosis of the tadpoles. Boiled

cabbage, boiled lettuce, boiled egg yolk, cooked goat meat, ripe banana and mixed food (combination of all food items). A qualitative and also a quantitative analysis was carried out

Qualitative analysis

The tadpoles fed with boiled cabbage, and lettuce attained a mean weight of 0.230g and 0.228g and metamorphosed by 36 to 38 days.

The tadpoles fed with mixed food attained a mean weight of 0.220g and metamorphosed by 39 to 40 days.

The tadpoles fed with boiled egg yolk and cooked goat meat attained a mean weight of 0.190g and 0.182g respectively and metamorphosed by 41 to 44 days.

The tadpoles fed with ripe banana died by the 27the day of growth, at which time their mean weight was 0.100g.

Quantitative analysis :

As boiled cabbage second favourable for growth of the tadpoles, its quantitative effect on growth and metamorphosis of tadpoles was examined. In all six quantities of boiled cabbage 0.1g, 0.2g, 0.4g, 0.6g, 0.8g and 1.0g were given to 5 tadpols/250ml of medium which was changed every two days.

The tadpoles reared with rations of 0.8g and 1.0g attained a mean weight of 0.228g and 0.220g respectively and metamorphosed by 37 to 38 days.

The tadpoles reared with a ration of 0.6g attained a mean weight of 0.230g and metamorphosed by 36-37 days.

The tadpoles reared with a ration of 0.4g attained a mean weight of 0.204g and metamorphosed by 40.-41 days.

The tadpoles with a ration of 0.2g and 0.1g attained mean weights of 0.150g and 0.140g and metamorphosed by 41-42 days Apparently no morphogenetic abnormilities were ween in the tadpoles.

DENSITY EFFECTS

(1) Density dependent growth and metamorphosis

The growth and metamorphosis of the tadpoles has been examined in isolation and at 7 densities of 4,8, 16,32,64, 128 and 256/2.5 lof medium.

In isolation maximum mean weight of 0.230g was attained just before metamorphic climax. This was followed by a sharp decrease in mean weight to 0.170g at froglet stage. Metamorphosis was complete by 36-37 days.

At densities of 4,8, & 16 the tadpoles attained mean weights of 0.228g, 0.230 g & 0.217g respectively and metamorphosed by 36-41 days.

The tadpoles reared at densities of 32 and 64 attained mean weights of 0.200g and 0.160g respectively and metamorphosed by 42-44 days.

The tadpolesreared at high densities of 128 and 256 attained a mean weight of 0.140g & 0.124g respectively. Tadpolesof density 128 metamorphosed by 56 days where as at density of 256 no sign of metamorphosis was seen till 56 days at which time the experiment was terminated.

(2) Effect of culture medium

With the change of culture medium every two days, the tadpoles attained a mean weight of 0.230g and metamorphosed by 36-37 days. When the culture medium was not changed, they attained a mean weight of 0.156g and metamorphosed by 50-52 days.

TEMPERATURE TOLERANCE

The analysis of temperature tolerance provided the following results.

(1) At room temperature ($\bar{x}$ 23^{o}C) the tadpoles attained a mean weight of 0.230 g and metamorphosed by 36-37 days. At high temperature of 32^{o}C, the tadpoles attained a mean weight of 0.185g and metamorphosed by 34-36 days. At low temperature of 10^{o}C , the development proceeded slowly and tadpolesattained a mean weight of 0.280g, but did not metamorphose till 56 days.

(2) Thermal tolerance and acclimation.

The critical thermal maxima (CTMax) and critical termal minima (CTMin) of the tadpoles at hind limb stages and Juvenile stages of SV length 28-30mm were determined.

The CTMax was found to be 41.5°C and CTMin was 5.9°C respectively. For the juvenilesand CTMax was 40.5°C and CTMin was 9.6°C.

The tadpoles acclimated at 10°C, had a mean CTMax of 37.2°C, while there acclimated at room temperature ($\bar{x}$ 23°C) had a mean CTMax of41.5°C. TAdpoles acclimated at 35°C had amean CTMax of 42.1°C.

(3) Temperature tolerance during development

A distinct pattern of changes in CTMax during the embryonic stages was observed. At stage 4 the CTMax was about 37.6°C and increased sharply to 41.8°C and at stage 22, it decreased to 41.1°C.

Similar changes in CTMax were seen at different larval stages. The tadpoles at stages 22-25 had a mean CTMax of 41.4°C and at stages 26-27 it was 41.5°C. At stages 28-29, the CTMax decreased to 40.6°C and at stages 30-31 it was 39.5°C. At froglet stages the CTMax recorded was lowest at 37°C. Juvenile stages again showed an increase till a CTMax of 40.5°C was reached at juvenile stages of SV length 28-30mm.

The CTMin at stages 22-25 was 7.6°C and at stages 26-27 it was 5.9°C. The juveniles of SV length had a mean CTMin of 10.0°C while the juvenilesof SV length 28.30 mm had a mean CTMin of 9.6°C.

(4) Thermal tolerance : Daily rhythms

The daily cycles of CTMax of the tadpoles also shows interesting variations. The mean CTMax was 40.6^{o}C at 0500 hr and increased sharply to 41.3^{o}C at 0800 hr. This was followed by a slight increase to 41.6^{o}C at 1100 hr. It remained almost unchanged till 1400 hr when a slight decrease to 41.5^{o}C was observed. The mean CTMax than further decrease to 41.0^{o}C at 1700 hr and to 40.8^{o}C at 2000 hr. Thus a temporal pattern of variations in CTMax were observed during the day.

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