CHAPTER I

INTRODUCTION
1. INTRODUCTION

1.1 Importance of Amphibians

The amphibians are of great importance to the human population as well as to the ecosystem. Frog oil has also medicinal value. Frogs and toads are an important carnivore consumer group and they eat insects, snails, earthworms, fishes etc. and they are eaten by snakes, birds, some mammals like mongoose and man. Hence, they form an important link in the food web in the aquatic ecosystem and terrestrial ecosystem. The complex food web in many natural ecosystems are maintained due to the presence of different species of frogs and toads. Anuran tadpoles also play an important role in Indian freshwater system during the monsoon period. The periodic presence of anuran larvae in fresh water in large numbers provide an excellent opportunity to examine the impact of transient consumers on the dynamics of aquatic ecosystem. Anuran larvae are an important link in the food chains of natural freshwater system, feeding on algae and planktons and being preyed upon by naiads and larvae of other aquatic insects, fishes etc. (Heyer and Muedeking, 1976).

Rates of material transfer within the ecosystem are influenced by temperature, light and other physical factors, and by biotic interaction (Seale, 1980). Nutrients and energy in material lost to biota, through excretion, death and predation, become available for assimilation by other organisms. This continuous, cyclic process sustains growth of all species present. Amphibians may be important regulators of nutrient cycling within ecosystems. Further more, they may transport material between terrestrial and aquatic ecosystems.
through migrations and dispersal after metamorphosis. In the "typical" complex life cycle of temperate amphibians, eggs containing energy assimilated by terrestrial adults are deposited into fresh waters where larvae accumulate material and energy and later metamorphose and transport this biomass back to land on dispersal.

Amphibians in general and ranids, bufonids and hylids in particular are considered to be indiscriminate predators on insects and worms (Tyler and Hoestenbach, 1979). They occupy an important position in the ecological pyramid and are known as "Farmer's friends". Anurans supplement the efforts of birds in killing insects and thus help in the maintenance of nature (Noble, 1931). Several important agricultural pests belonging to Orthoptera, Isoptera, Lepidoptera, Hemiptera and Coleoptera form the major bulk of the food organisms of frogs. Thus frogs and toads do serve as an important controlling agent of agricultural pests. Frogs do also consume a good percentage of crabs which destroy the agricultural land by their burrowing activity.

Frogs and toads are used in biology laboratories, medical and pharmaceutical laboratories as the biological specimen for vertebrate type for dissection. As these animals are easily available, defenceless and their organ systems are primarily very similar to mammals and man, they are used as class room material for the beginners. The syllabus of almost all Indian universities demands dissections of at least ten frogs by a biology student prior to his/her graduation.

Frogs and toads are used for production of "frog oil" and "frog meals". Frog oil contains medicinal properties and is used in leather
industries and frog meal is used as poultry and cattle feed. Frog legs serve as delicious food for the rapidly growing human population. Toad skins have been used as a source of fine leather.

Being the first tetrapods, anurans are also important for evolutionary study of land vertebrates and are regarded as the connecting link between the aquatic fishes and first true land vertebrates, the reptiles.

Scientists have used amphibian tadpoles as a tool to understand various problems of differentiation and development (Barth and Barth, 1967 and Kollros, 1968). Our knowledge of the basic principles of developmental biology is largely based on the gross and experimental studies of the amphibian eggs. Being easy to get in large numbers and to maintain in the laboratory conditions, eggs provide important material for a developmental biologist to work with. Frog eggs also serve as food for the aquatic larvae.

1.2 Declining of Amphibian populations

There has been a recent spate of interest in world-wide declines in amphibian populations (Barinaga, 1990; Milstein, 1990; Phillips, 1990; Wyman, 1990 and Wake, 1990). Observations indicate that some amphibian populations have declined in size, while others have become extinct, have recently received wide spread attention from both biologists and the news media. Prior to 1989, many herpetologists were aware of population declines or extinctions in their immediate geographical area, but not until a workshop on the subject was organised under the auspices of the National Research Council did the world-wide scope of the problem
become clear (Barinaga, 1990; Phillips, 1990). In many cases the declining species are not those that have been designated as “threatened”, but in stead are those that were formerly represented by large populations. The global scope and the rapidity with which these declines and extinctions have occurred have led to the conclusion that anthropogenic causes must be largely at fault and that a single environmental factor is not likely to have caused such wide spread dieoffs (Phillips, 1990). The demise of each population could be caused by one factor or by synergistic effects of two or more factors which are as follows -

i. Pathogens

The bacterium *Aeromonas hydrophila* is linked to amphibians septicemia, a pathogenic condition commonly described as “red leg”. These bacteria found ubiquitously in fresh waters, infect fish, amphibians, reptiles and even humans. Since *Aeromonas* is present in fresh waters and is found in and on healthy amphibians, the disease is understood to develop when an individual becomes immune suppressed, or when its immune system loses the ability to prevent infection by the bacteria. *Aeromonas* is present on the skin and in the digestive tract of healthy amphibians (Hird et al., 1981). Newly infected individuals stop eating and as the infection spreads septicemia develops and capillary dilation of the skin of the abdomen and ventral surfaces of the legs occurs, followed by haemorrhages which give rise to a reddish skin, hence “red-leg”. Mass mortality of adult *Rana muscosa* in Sierra Nevada of California (Bradford, 1991), adult frogs (*Rana pipiens* and *Acris crepitans*) and Salamanders (*Eurycea neotenes*) in Texas (Hunsaker and Potter, 1960), has been attributed to “red-leg”. Although frog
septicemia has been reported from several wild ranid frog populations (Lee and Franz, 1973; Hird et al., 1981) and may become epidemic among captive frogs (Glorioso et al., 1974), the influence of septicemia associated bacteria on wild ranid population is controversial. “Red-leg” symptoms are also linked to bacteria other than *A. hydrophila* as well as to simple wounds (Gibbs, 1973).

**ii. Acid rain**

Acid rain is an interesting possibility of declining of amphibian populations. Acid rain has been implicated in lowered pH of temporary pools, probably causing embryonic salamander mortality in northeastern United States (Pough and Wilson, 1977). Ranids could be affected by decreased pH through developmental anomalies (Tome and Pough, 1982; Pierce et al., 1984) reduced sperm mortality and thus lowered fertilization effectiveness (Schlichter, 1981), interference with ion regulatory capabilities similar to those found in fish (Fromm, 1981; Freda and Dunson, 1985), or decreased growth rates (Freda and Dunson, 1985). In the laboratory, *R. catesbeiana* embryos appear more acid sensitive than embryos of species of more closely related to *R. aurora* and *R. boylii* (Tome and Pough, 1982). The pH of breeding ponds in the Colorado Rockies has not decreased to lethal levels to cause extinction or decline of the population of *Rana* and *Bufo* (Corn et al., 1984; Corn and Vertucci, 1992) but only just enough to cause stress and disease. Research on responses of amphibians to pH have largely concentrated on tolerance of amphibian eggs and larvae to reduced pH (Tome and Pough, 1982; Freda and Dunson, 1985; Pierce, 1985). Lethal pHs vary from 3.0 (*Xenopus laevis*) to 5.0 (*Ambystoma maculatum*).
iii. **Temperature**

Temperature plays a synergistic role by hampering a potential immunological response to an infectious threat. Low temperature appears to compromise the ability of the immune systems of ectothermic animals to combat disease. Montane populations of *Bufo canorus*, *Rana cascadae*, *Rana pretiosa*, *Rana pipiens* and *Rana muscosa* have disappeared from the Sierra Nevada Range in California and in some cases these species are extinct over portions of their former ranges (Phillips, 1990; Bradford, 1991). Montane amphibians not only spend the entire winter at body temperatures just barely higher than freezing, but they also spend reasonable amounts of time at cold body temperatures during the night and on cloudy days during their active season (Carey, 1978). *Aeromonas hydrophila* and a few other pathogenic bacteria are common in the intestines of amphibia at low temperatures (Carr *et al.*, 1976). Therefore, the probability of successful infection of immuno suppressed animal is good at low temperatures at which an immune response may be limited or non-existent. It is interesting that the major die-offs occurred during the winter in Montane *Bufo boreas boreas* populations.

iv. **Toxicants**:

A diverse array of compounds (pesticides, herbicides, heavy metals, and others) are known to have deleterious effects on various life stages of ranid frogs (Hall and Swineford, 1980; Mohanty-Hejmadi and Dutta, 1981). Field observations suggest that toxicant-induced mortality among North American ranid frogs are limited (Ferguson, 1963; Meeks, 1968). Larval stages appear to be more
sensitive to toxicants than are embryonic and post-metamorphic stages (Meeks, 1968). Hyperactivity and prolonged pre-metamorphic development are sublethal responses which can increase vulnerability to predation (Yeung, 1978). Therefore, toxicants in one hand may have been responsible for ranid frog mortality, but since the use of at least chlorinated hydrocarbons (DDT etc.) postdates the period of significant decline in at least one species (e.g. *R. aurora*) by at least 30 years, it is unlikely that those toxicants were responsible for the noted decline.

Embryopathic effects of lead in *Microhyla ornata* was studied by Ghate (1985) which suggests that lead was observed to induce specific abnormality in the embryos at a concentration of 20 ppm and above, while 40 ppm concentration was highly embryotoxic. The effect of a common pesticide “Rogor” in Orissa on the life cycle of *Rana tigrina* was determined by exposing eggs and larvae to different concentrations (Dutta and Mohanty-Hejmadi, 1978). “Rogor” was found significantly more toxic to pre-metamorphic larvae than to eggs. The pesticide affected survival, length of larval period and size at metamorphosis when introduced at early stages of development.

v. *Catastrophic Mortality* :

Sweet (1983) suggested that declines of *R. boylii* were the result of scouring floods that occur about once every 500 years, whereas Corn and Fogleman (1984) implied that severe drought may have been responsible for the local extinction of *Colorad R. pipiens*. Bradford (1983) indicated that during some winters, almost all post-
metamorphic *R. muscosa* in shallow lakes die, probably because of oxygen depletion.

**vi. Ultraviolet radiation:**

Ultraviolet radiation may be implicated in amphibian declines. UV radiation can encourage the development of malignant tumours, and it may inhibit the activity of T-suppressor lymphocytes (Longstreth, 1990). Because amphibians are poikilotherms, some bask in the sun to warm themselves, and many species undergo embryonic development in sunlit waters. The thin epidermis of amphibians may be especially sensitive to UV radiation.

**vii. Fish predation:**

Introduction of transplanted and exotic fishes are responsible for ranid declines (Hammerson, 1982). Grinnell and Storer (1924) comment that local declines of *R. muscosa* were known to be caused by trout (*Salmo* spp.) and Charr (*Salvelinus* spp.) introductions. The fish hypothesis assumes that frog declines are caused by predation. Voris and Bacon (1966) concluded that fish are more effective than most preying organisms at preying on tadpoles; Heyer *et al.*, (1975) amplified that conclusion, noting that fish are the only purely aquatic predators capable of eliminating anurans from pond systems. The salmonids occasionally prey upon frogs or tadpoles (Calef, 1973). Tadpoles of species infrequently exposed to predators have been shown to be highly vulnerable, either because of their greater activity (Woodward, 1987) or palatability (Kruse and Francis, 1977) but may also be vulnerable because of an inability to escape novel predators (Minkley, 1983; Meffe, 1985). Lewis and Helms (1964) found that ranid larvae were more vulnerable than fish to fish
predation, which may result from the more limited stamina tadpoles exhibit (Wassersug and Feder, 1983) because the mechanical efficiency of tadpole locomotion does not differ significantly from that of fishes (Wassersug and Hoff, 1985). Introduced centrarchids (Sun fishes) may be more maneuverable than native salmonid predators (Webb, 1983), but whether ranid tadpoles, highly maneuverable swimmers (Wassersug and Hoff, 1985), are at greater risk of predation by centrarchids. Eggs may even comprise a substantial portion of the diet of the less piscivorous species (e.g Lepomis macrochirus). Anuran eggs are rarely reported as prey (Pearse, 1915-16). Moyle (1973) reported a negative relationship between R.boylii numbers and fish abundance and noted that the fish fauna at sites with bull frogs tended to be dominated by the introduced fishes Lepomis cyanellus and Gambusia affinis.

viii. Environmental pH:

General circulation models have been used by climatologists to predict how the earth’s climate may respond to increasing concentrations of atmospheric green house gases (Wyman, 1990). These models suggest that temperature regimes will change, with greater warming away from the equator. They also agree in general that continental interiors will be drier and that droughts will be more frequent and more intense (Wetherald, 1991) which will lead to the reduction and elimination of populations living at the extremes of their physiological capability. The hypothesis support that global climatic change may be playing a role in the declines. Aquatic life stages of amphibians are sensitive to acidic conditions. Pierce (1985) reported that with the exception of Xenopus laevis with a tolerance of pH 3.5, all the rest 24 species of frogs and 4
species of salamanders have tolerances of pH 3.7 or greater. The pH of most aquatic habitats generally fall between a pH of 4 and 6. The temporary acidification of ephemeral ponds and streams early in the spring following snow melt may be partially responsible for the decline in some *Ambystoma tigrinum* populations. At pH 3 and below it, development was immediately arrested and the embryos of *Microhyla ornata* were killed within a few hours (Padhye and Ghate, 1988). *Ambystoma jeffersonianum* and *Bufo woodhousei* embryos were intolerant to low pH and were absent from the most acidic ponds (Freda and Dunson, 1985). Water of low pH stops development of embryos or prevents hatching due to failure of the vitelline membrane to expand (Freda and Dunson, 1985). Compounds of unknown composition in bog water and cations such as Na, Mg, Ca and Al can influence the sensitivity of embryos of amphibians and fish to low pH (Freda and Dunson, 1985).

ix. Predation and Competition:

Moyle (1973) suggested that bull frogs might eliminate native ranids by either predation or competition. Few data suggest that predation by bull frogs might be responsible for ranid frog decline. *Rana catesbeiana* unquestionably pursue (Stewart and Sandison, 1972) and eat post-metamorphic life stages of other anurans (Applegarth, 1983). Laboratory studies show that *Rana tigerina* tadpoles predate on larvae of *Bufo melanostictus* (Hota and Dash, 1983). Larvae of some species of *Ceratophrys* and *Rana* also show cannibalism. These factors deplete their population in nature.

Competition among amphibians is associated with resource limited conditions frequent at high population densities (Wilbur,
1980; Smith, 1983). Although the greatest potential for competitive effects exist in denser larval environments, high larval mortality seems to prevent densities from reaching the levels necessary for competitive effects to be significant (Calef, 1973; Dash and Patra, 1977; Dash and Hota, 1980; Hota and Dash, 1981; Mishra and Dash, 1984; Dash and Dei, 1996).

Water snakes and garter snakes devour many of the smaller amphibian species. The exotic snakes include a very high percentage of toads in their diet (Noble, 1931). Besides the snakes, other reptilian enemies of amphibia include aquatic turtles. Birds and mammals take a very high toll of amphibian life. Domestic ducks can clean a pond abundant with tadpoles in a very short time. The common crow eats frogs and toads more regularly than any other kind of food. Owls are also known as the predators of many amphibians. Some mammals like weasels, skunks and even rats predate on amphibians.

x. Habitat alteration:

The habitat alteration hypothesis implies that human induced habitat alteration have reduced or eliminated populations of endemic *Rana*. Certain physical aspects of the habitat are critical to survival of frog populations and that their modifications or removal reduces or eliminates those populations. Banta and Morafka (1966) suggested that the destruction, artificial drainage, and cleaning of natural ponds were the types of alterations that eliminated the endemic *Rana* populations. For *R.boylii*, oviposition site is determined by the presence of a solid substrate in flowing water (Storer, 1925; Zweifel, 1955), and this species is absent from sites
that lack such substrates (Fitch, 1938). Similarly, populations of *Rana aurora draytonic* are associated with deep-water pools fringed by thick vegetations (Zweifel, 1955) which may provide a refuge from predation or from unfavourable environmental conditions for post-metamorphic individuals, specially adults.

**xi. Habitat Fragmentation:**

Habitat fragmentation may also reduce or eliminate amphibian populations because smaller habitats support fewer species. The fragmentation and isolation of habitats cause the dispersal movements which result in the loss of many isolated populations over time. Even relatively undisturbed habitats may be fragmented to amphibians because the forest interior is more acidic than streams and ponds. These acidic conditions may prevent amphibians from moving among aquatic habitats. Because many amphibians must migrate between aquatic and terrestrial habitats to reproduce and because those habitats in many places are separated by roads, migrating amphibians also vulnerable to automobile traffic. The Western Ghats of Southern India are rich in amphibian fauna with 117 species of which 89 species (70%) are endemic to the geographic area. But the fragmentation of rain forests of amphibian fauna of the Western Ghats may lead to the decline of amphibian population. In the present study of *Microhyla ornata* which inhabits in the rice fields and breeds in temporary pools nearer to their habitats, the number of breeders in the site of oviposition decreased when the rice fields were fragmented into small plots (Dei, personal observation).
xii. Forest soil acidity:

Thin high-elevation forest soils overlying bedrocks low in acid neutralising capability are also acidic, and these soils may not be able to neutralise the effects of high inputs of sulphuric and nitric acids. In some of these soils the pH is driven down to an equilibrium point of pH 4.2 by inputs of sulphur and nitrogen inputs then depress the pH to below 4. Thus, the potential exists for acidic deposition to reduce the final equilibrium pH of forest soils to lethal conditions for amphibians that live in intimate contact with those soils. Forest soils become even more acidic when they dry during a drought. The effect is particularly strong in conifer forests, soil pH routinely falls to below 3.7 during dry periods. Very few amphibians are found in spruce forests. During dry periods salamanders living on acidic substrates are faced with a multiple challenge of dessication and osmotic problems caused by reduced soil pH. Two threatened small salamanders _Desmognathus wrightii_ and _Plethodon nettingi_ appear to be at the extreme of their physiological tolerance for pH because they are small, making the energy demand for osmoregulation higher for them than for larger salamanders. The increased frequency and intensity of droughts in the recent past, combined with existing acidic conditions and with the synergistic effects of low soil moisture, low pH and high temperature may be pushing them towards extinction.

However, many amphibian species are capable of surviving in acid solutions of pH 4 - 4.5 (Pierce, 1985), but it is important to consider the acidity of the habitats relative to the species tolerance limits. Finally some variation in size of amphibian populations certainly has natural causes (Shoop, 1974; Pechman _et al._, 1991).
Clearly, we need much more data on past and present densities and distributions of amphibians. Research must be aimed at identifying where populations are healthy and where they are not, and how environmental conditions differ between the two places. These studies will have to be interdisciplinary because of the complexity of the phenomena involved. The potential for synergistic interactions among a host of environmental parameters make the experimental elucidation of cause and effect extremely difficult. However, future research concerning the proximate and ultimate causes of declines of amphibians from pristine habitats should address synergism among possible factors. Only through long-term monitoring programmes and carefully controlled field experiments can we determine whether amphibians are signalling environmental degradation or whether other factors are affecting population dynamics of amphibians.

The Coimbatore Zoological Park and Conservation Centre is actively pursuing conservation of some recent grants helping the zoo realise its initial dreams. Under the Jersey Wildlife Preservation Trust's grant scheme under Gerald Durrel Memorial Fund, the Trust has awarded project grants to eight of its trainees around the world. Coimbatore Zoo has been awarded funds for two projects - Amphibian Conservation and Education Project and Invertebrate Conservation and Education Project. The amphibian project is well underway, having started in January, 1977. The main objectives of the project are:

a. Identify local species at the Zoo site for captive husbandry studies and breeding.
b. Survey of zoo site and adjoining areas in the Nilgiri Biosphere Reserve of the Western Ghats for amphibian diversity.

c. To develop interpretive graphics relating to amphibians at the zoo.

d. Create awareness among the locals and general public including students in schools and colleges in Coimbatore.

e. To develop educational packages.

Till date 14 species have been recorded during the dry months of the year and more species are likely to be found during the surveys in the monsoon period which commences in Coimbatore in the month of July. Of 14 species, 2 are endemic to the Western Ghats. Species behaviour is being studied in captivity provided with substrates similar to the species habitat in the wild. The technique of keeping still needs refinement but fortunately there have been no mortality among the captive amphibians.

The programme of keeping and breeding amphibians in captivity will continue as the main goal of the zoo which will lead to the preservation of species which are on the verge of declining or extinction.

1.3 Culture, Care and Maintenance of Indian Frogs:

The amphibians occupy an unique position in ecosystems because on one hand they control insect pests and on the other hand act as prey for other large animals. They are an important link in terrestrial as well as freshwater aquatic ecosystems. A decline in amphibian populations can have an adverse effect on the whole of
the ecosystem. Based on a field study of food habits, Issac and Rege (1975) and Abdulali (1985) have reported that *Rana tigerina* plays a significant role in controlling agricultural and other pests in the field. Analysis of stomach contents of six species of frogs (*Rana breviceps*, *R. cyanophlyctis*, *R. limnocharis*, *Uperodon systoma*, *Microhyla ornata* and *Polypedates maculatus*), it is reported that there is partial selectivity in feeding which is based on the ecological niche, each species occupies (Mohanty-Hejmadi and Acharya, 1982). In addition, an “All India Co-ordinated Marine Products Export Development Authority (MPEDA) Project on Survey of Frog Populations” from some of the Indian States i.e. Kerala, Maharastra, Gujarat, Andhra Pradesh, Orissa, West Bengal and Karnataka have shown that insects and their larvae do form the major constituents of the food of juvenile frogs weighing less than 50 g. of body weight. The contributions of insects and their larvae were reduced to 32 % and further to 2% in frogs weighing 50 to 100 g and more than 100 g respectively. The large adult frogs feed on an astonishing variety of small and large prey invertebrates, including snails, crayfish, terrestrial and aquatic insects and rachnids, along with vertebrates including other amphibians, reptiles, birds, small mammals and some plant materials. Amphibian predators may subsist on simple insect diets: house crickets (*Acheta domestica*), mosquitoes (*Culex pipiens*), fruit flies (*Drosophila melanogaster*) and other flies. Some capture earthworms (*Lumbricus* spp.), tubifid worms (*Tubifex tubifex*), and soft-bodied insect larvae, such as *Tenebrio* (mealworm) larvae. Aquatic amphibian predators may take live mosquito fish (*Gambusia affinis*), (Modzelewski and Culley, 1974) or commercial brine shrimps. Crabs, earthworms and tadpoles formed major (86%) constituents of food of large frogs. Therefore,
releasing of juvenile frogs into the paddy fields will be a useful programme for completely realising the limited role played by frogs in pest control (MPEDA report, 1989).

India used to be one of the leading exporters of frog legs from 1980 - 1985; but Government of India enforced a ban on export of frog legs during 1987 as it was alleged by farmers and environmentalists that frog collection upsets the ecological balance in agricultural and aquatic ecosystems. This was because most of the frogs were collected from nature and no attempt had been made for mass culture of frogs.

Studies on the scientific frog culture in India has been initiated very recently by Central Inland Fisheries Research sub-station, Cuttack, Orissa (Das et al., 1976). Mohanty-Hejmadi (1974a) discussed about the maintenance of anuran juveniles and adults in the laboratory. Dutta and Mohanty-Hejmadi (1992) have been working on several species of aquatic, terrestrial and burrowing species of amphibians and have standardised the techniques for mass culture of frogs of 3 aquatic species (*R. cyanophlyctis*, *R. hexadactyla* and *R. talpehensis*), 3 semiaquatic species (*R. crassa*, *R. tigerina* and *R. limnocharis*), 4 burrowing species (*Microhyla ornata*, *Ramanella variegata*, *Tomopterna rolandae* and *Uperodon systoma*).

The culture methods include:

i. Collection of amplexing pairs from their breeding grounds during rainy seasons.
ii. Maintaining the amplexing pairs in the laboratory for egg laying.

iii. Large scale hatching of fertilized eggs in the laboratory.

iv. Releasing of the froglets into suitable natural environment for growth in nature.

v. Rearing of males and females in captivity.

vi. Obtaining of fertilized eggs by induced ovulation and artificial fertilization.

The captive rearing of froglets after metamorphosis is as follows -

i. Captive rearing of froglets of *Uperodon systoma*, *Ramanella variegata*, *Microhyla ornata* and *Tomopterna rolandae* has been made possible by feeding the froglets with termites. This captive rearing is possible in the laboratory by keeping the froglets in aquaria containing sand, as the above cited species are burrowing in nature.

ii. Froglets of aquatic species like *R. hexadactyla*, *R. cyanophlyctis* and *R. talpehensis* can be reared in captivity in artificial ponds with suitable fencing by wirenets. Limited number of froglets can be reared in such ponds to assess their growth. Once the froglets attain a desirable size, they can be marked by toe clipping to record individual growth which will provide a basis of obtaining data on sexual maturity and size at first reproduction. The above methodologies are some of the important parameters for obtaining large scale froglets for subsequent release into suitable environment which is one of
the ways to increase the natural population of desired species of frogs.

India having numerous irrigation channels, offer excellent ecological niches for the successful survival and rapid multiplication of amphibians. Our environments provide wide scope for the culture of frogs. For perfect laboratory culture of frogs and toads, knowledge on their biology, anatomy, physiology, ecology, energetics, etc. are highly essential.

Most current attempts to frog culture in U.S.A employ outdoor facilities and the only economical market has been through the sale of these pond-reared frogs for pond-stocking purpose or breed-stock (Priddy and Culley, 1971). Gibbs et al., (1971) reported about two pioneering efforts to raise and maintain frogs by John Priddy (Southern Frog Farm, Dumas, Ark - 71639), Dr. Culley (Louisiana State University, Department of Forestry. and Wild Life Management, Baton Ruge, La). Nace (1968), Director of Amphibian Facility at the University of Michigan, maintains colonies of different frog species in his laboratory. Dr. T.Kawamura, former President of University of Hiroshima, maintained species of Ranidae, Hylidae and Discoglossidae successfully through many generations, out of which some strains have now been in the laboratory for about eight generations.

Brief accounts of frog culture in Japan are available. Breed stocks of the American bull frog, Rana catesbeiana were shipped to Japan from the United States during the early 1900's. The basic rearing techniques involved confinement and concentrated feeding with fly larvae, silk-worm pupae, cray fish etc.
Recently frog culture has been included as a branch of aquaculture and scientific researches are in progress throughout the world to find out a suitable culture method for the frogs and toads. There is vast scope for adoption of standard procedures like hypophysation, dry fertilization and mass rearing of tadpoles in high density cultures using tubifex as food. The most crucial problem on mass culture of tadpoles and adults is to find a live and kicking prey in adequate quantity. Recently Marian and Pandian (1984, 1985) have described a simple procedure to mass culture of tubifex using waste organic matter. Reared solely on *Tubifex tubifex*, hatching of *Rana tigerina* attained a body weight per larva of 40 g in 90 days at the feed cost < 200 g tubifex (Marian, 1982). This is approximately the suitable size for release into the paddy fields to ensure conservation, and pest control.

In the recent years education sector of India has become an important user of frogs and utilizes about 18 million frogs/annum. Therefore, the need for aquaculture of frogs has been emphasized repeatedly (Culley, 1973). However, frog culture has been found not a very successful enterprise (NAS, 1974), the causes for this area (i) incidence of heavy mortality of tadpoles owing to cannibalism (Marian, 1982; Hota and Dash, 1983; Marian et al., 1985) (ii) the obligate requirement of proteinaceous feed for the grown-up tadpoles (iii) the requirement of live ‘kicking’ prey for the adult prey (Nace, 1977). In India, Mondal (1975) attempted to mass culture frogs by providing illumination to attract phototropic insects. Aquaculture of tadpoles may ultimately serve two purposes:

i. One for ranching programme and
ii. Harvesting for edible purpose.

The former involves mass rearing of tadpoles and release of juveniles into the irrigated field areas, which will help in controlling the agricultural insect pests. On the other hand, mass culture of large frogs in aquaculture farms will be required for scientific education and export purposes. Both programmes require much scientific and Governmental support, and operation of these programmes in a judicious proportion may provide maximum benefits.

Care and Maintenance of Juveniles and Adults:

It is necessary to use animals bred and maintained under controlled conditions for obtaining comparable results in biological experiments. Because of the popularity of amphibians both for research and teaching, frogs are being raised under standardised conditions in U.S.A. and Japan. In India also, amphibians are used extensively for research and teaching and therefore an attempt was made to raise several local amphibians (Mohanty-Hejmadi, 1977) for varying length of time. The following are some of the criteria which were found to be optimal for the maintenance of the juveniles and adults.

1. Housing:

Glass aquaria of different sizes are most suitable for maintaining frogs as they are non-corrosive and rust proof. Suitably moulded heavy duty plastic containers are used at the Amphibian Facility of the University of Michigan, U.S.A (Nace, 1968). In India, glass aquaria are arranged in a row on a shelf at a convenient
height so that the animals can readily be observed. Generally, aquaria of three sizes such as -

1. **large** - 24” x 12” x 12” of approximately 57 litre capacity.

2. **Medium** - 18” x 10” x 10” of approximately 30 litre capacity.

3. **Small** - 14” x 8” x 10” of approximately 18 litre capacity are used. Aquaria larger than these are difficult to carry and clean. The contents of the aquaria depend on the frogs to be housed in. The number of frogs per aquarium depends on the size of frog as well as size of the aquarium.

Aquatic *Rana cyanophlyctics*, the skipper frog can be maintained in an aquarium filled up to 12 cm with water. Since these frogs love to dive into the mud when disturbed, the bottom may be filled with 5 cm of sand. The aquarium should be cleaned twice a week. For *Rana tigerina*, the Indian Bull frog, an amphibious environment is necessary. This is achieved by filling one side of the aquarium with clean sand up to a height of 15 cm. The other side of the aquarium is filled with water. To prevent the spreading of sand into water, a piece of wood of suitable height can be placed in between sand and water. A few pieces of broken flower pot should be placed on the sand to provide a convenient terrestrial area for feeding as well as hiding. The aquarium should be cleaned twice a week. This set up is also satisfactory for cricket frog *Rana limnocharis*. 
For the Tree frog Polypedates maculatus, the aquarium should be filled up to a depth of 12 cm. with wet sandy soil and introduction of a variety of plants like Colocasia that keeps the frogs under natural conditions. A large finger bowl should be sunk into sand in such a way that its edges are either covered or flush with the level of sand and this finger bowl should be filled with water. Since these frogs like to sit near the top of the aquarium, a folding non-perforated metal top in stead of a sliding one should be provided. The aquarium should be cleaned once a week. A similar set up with a wire meshed top is suitable for Bufo melanostictus.

For Microhyla ornata, an amphibious setting like that of Rana tigerina is satisfactory, however, these frogs love to hide under suitable covers and a pyramid made up of broken flower pots are ideal for them. The aquaria are cleaned once a week.

For burrowing forms like Rana breviceps, Ramnella variegata and Uperodon systoma, aquarium should be filled with wet sandy soil up to a length of 12 cm. A corner of the aquarium should be filled with water which provides an aquatic environment and keeps the soil damp. Cleaning of the aquarium once a month is satisfactory as these frogs need very little care.

II. Water:

Although a steady flow of water into and out of aquaria can reduce the time necessary to clean these tanks, under local conditions where the turbidity and pH of water varies greatly, it is preferable to keep frogs in standing water. Without provisions for automatic dechlorinating device and pH tester etc, a running water system can become disastrous to frogs. Besides, most frogs live in
standing water or near water, therefore maintaining them in running water can introduce undesirable physiological stress. Under local conditions it is best to store tap water in large tanks fitted with outlets at about 10 cm to 15 cm above the bottom. Storage of water not only conditions it but allows the suspension in the turbid water to settle down to the bottom of the container, below the level of the outlet. By attaching suitable length of plastic or rubber pipes fitted with clamps, the aquaria can be filled easily. Plastic or rubber pipes can also be used for draining the aquaria.

III. Food:

Providing food is the biggest problem for raising amphibians. Most of them eat live insects and therefore, at the Amphibian Facility of the University of Michigan (Nace, 1968), several populations of insects are maintained to provide food for the frogs. Under local conditions, getting chunks of termite hill once a week is satisfactory. Since the termites can be kept in the laboratory in convenient cans without much effort, this is standard food in the laboratories. Although an exclusive termite diet has proved to be adequate for Microhyla ornata, Ramnella variegata and Uperodon systoma, other species need larger insects in the diet. Since Microhyla loves to crawl into termite nests, care should be taken to examine empty portions of termite nests after feeding. During the rainy season, leaving bright light above large pans of water proved to be a good method for trapping insects. At other times insects have to be collected manually. This insect diet is supplemented with earthworms. At present, attempts are being made to raise crickets, earthworms and fishes in the laboratory to feed the frogs. Food can be given in the morning to all species except the burrowing ones.
The burrowing ones are nocturnal in habit and therefore, they should be fed in the evening. The amount of food necessary to saturate each group can be determined with little effort.

iv. Health:

When collecting frogs from nature, the “sick looking” ones should be discarded. The healthy ones should be given a wash in potassium permanganate solution. The solution can be prepared by adding just enough powder to make the water light pink. Laboratory frogs should be checked once every morning and any sick looking frog should be isolated and put in a tank containing permanganate solution up to a height of 4 to 5 cm. For severe cases, chloromycetin in dosage recommended by Nace and Richards (1972) should be injected into the coelomic cavity. The most severe disease for frogs is the “red leg” disease which is caused due to haemorrhage and is usually fatal. This disease is caused by the bacterium *Aeromonas*.

1.4 Food and Feeding Habits of Anuran Larvae:

All known members of class Amphibia, at least as adults are primarily carnivorous (Reeder, 1964). But as larvae, however, amphibians vary widely in feeding habits, from strict herbivore through omnivore to carnivore. Moreover, the manner of acquiring food, and its subsequent digestion and assimilation are also variable. Noble (1931) mentioned that “tadpoles in general show greater food preferences than adult frogs and toads, fore some are carnivorous, while majority prefer a mixed diet”.

Anuran larvae hatch with large yolk reserves and typically do not feed for several days. The larvae of a few anurans have
sufficient yolk for all development and do not feed before metamorphosis, such as microhylids *Kalophrynus pleurostigma* (Inger, 1966), the laptodactylids *Eupsophus roseus* and *E. vittatus* (Formas and Vera, 1980) and the bufonids *Pleophryne alboteniata* and *P. lighti* (Inger, 1954). Most anuran larvae progressively absorb the nutrients of the embryonic sac before or shortly following hatching, and species with aquatic free living larvae have an early developmental period characterised by suspension (filter) feeding, the removal of suspended bacteria, zooplankton, phytoplankton, and organic detritus from inspired water (Seale, 1987). Suspension feeding anuran larvae feed continuously (Jenssen, 1967) on microscopic particles that are 3 or 4 orders of magnitude smaller than themselves and comparatively evenly distributed in the environment (Seale *et al.*, 1982). Many larval anurans have adaptations for removing particles from dilute suspensions (Wassersug, 1980). They are efficient and versatile suspension feeders capable of ingesting particles ranging in diameter from < 0.1 (Wassersug, 1972) to 200 μm (Seale, 1980). Both obligate and facultative suspension feeders occur in anuran tadpoles (Sokal, 1977; Wassersug, 1980; Seale, 1982). Larvae of *Xenopus laevis* are obligate mid-water suspension feeders, whereas larvae of genus *Rana* are facultative suspension feeders (Gradwell, 1975; Wassersug, 1980). The morphology of feeding apparatus, the oral cavity of different anuran larvae have been described and compared by a number of workers. The filter apparatus of different anuran families (*Hylidae, Bufonidae, Leptodactylidae, Ranidae, Microhylidae, Phyllobatidae, Papid, Rhinophrynidae, Pelobatidae* etc.) have been studied by (Kenny, 1969; Wassersug and Rosenberg,
1979). Savage (1952) also described the morphology of mouth parts of some anuran species. Viertel (1982) described the morphology of oral cavity of some central European anuran larvae and discussed the habit, oral cavity and filter feeding of *Baleaphyne muletensis* larvae. Sahu (1993) gave a detailed description of oraldisc, oral papillae, rostrodonsts, keratodonts in the tadpoles of five anuran species. Macrophagous feeding replaces suspension feeding as development proceeds in many anurans. Keratinized plates on the beaks scrape algae, bacteria, and other material from the substrate (Wassersug, 1980). In other anurans, however, suspension feeding remains the dominant or exclusive form of food acquisition until metamorphic climax. The development of branchial filter plates in different species is correlated with the degree of dependence upon suspension feeding (Savage, 1952; Wassersug, 1980). Well-developed filter systems are found in species using suspension feeding include the hylids *Agalychnis callidryas* and *Phyllomedusa trinitas* (Kenny, 1969), most microhylids (Savage, 1952), *Xenopus* (Wassersug, 1972, 1980; Wassersug and Rosenberg, 1979) and free swimming *Pipa* (Sokal, 1977).

Filter feeding and macrophagous collection of primarily plant material from the substrate are the two most common modes of feeding in anuran larvae, other patterns also exist and are generally associated with a reduction of the branchial basket. Several species like *Microhyla heymonsi* (Microhylidae), *Megaphrys minor* (Pelobatidae) have larvae with dorsally upturned rather than ventrally oriented mouths. These so-called funnel mouth larvae feed at the water surface on larger food particles. Carnivorous larvae like *Hymenochirus* (Pipidae) and *Anotheca* (Hylidae) feed on large
individual prey items, including pelagic arthropods found in either the water column or on the substrate. Marsupial frog larvae (Hylidae) apparently feed on exudates from the incubatory integument in the pouch. Many anuran larvae are cannibalistic (Noble, 1931; Ruibai and Thomas, 1988). One of the most interesting forms of “Carnivory” involved oophagy the “cannibalistic” consumption of eggs of the same species. This practice is common in anuran species with free-living arboreal larvae, including more than a dozen species in the Hylidae, Rhacophoridae, Microhylidae and Dendrobatidae (Lannco, Townsend and Wassersug, 1987). Carnivorous larvae tend to have shorter guts and hypertrophied jaw muscles when compared with omnivorous or herbivorous forms (Wassersug, 1980).

Several parameters may be of interest in evaluating a given food type of feeding schedule, growth in body mass and length, net protein synthesis, skin condition, reproductive capacity and resistance towards infections. The quality of the food depends not only on the content of nutrients, but also on its ability to elicit feeding and on its digestibility. Several workers have experimented on the diets of tadpoles to be used in the laboratory for successful metamorphosis. Mohanty and Dash (1986) tested the effects of five diets on growth and metamorphosis of larval Rana tigerina, Nagai, Nagai and Nishikawa (1971) fed artificial food or larvae of their own species to Bufo vulgaris, Scorgie (1980) tested four different diets on Bufo bufo larvae, Claussen and Layne (1983) fed Bufo woodhousii with mealworms, cabbage loopers, crickets or a combination of these and Modzelewski and Culley (1974) measured growth in Rana catesbeiana by feeding mosquito fish, earthworms,
crickets and their combinations. Hota and Dash (1981), Mishra and Dash (1984), Mahapatro and Dash (1987) fed the anuran larvae with mixed diet of *Amaranthus*, minced goat meat, boiled egg yolk in a ratio of 5:1:1 for the study of growth, metamorphosis and energetics. The intestinal contents of tadpoles of six anuran species were studied which revealed that the tadpoles were largely herbivorous and ingested 36 genera of algae and the type of food was determined by the nature of habitat (Sekar, 1992).

The tadpoles can ingest epiphytic algae (Dickman, 1968); epibenthic algae (Calef, 1973). algae in their own fecal pellets (Steinwascher, 1978a); suspended algae and detritus (Seale and Wassersug, 1979), organic particles associated with sediments (Seale, 1980) and with the air-water interface (Goldacre, 1949). Tadpoles can “shift” substrates in response to changes in food availability (Seale, 1980). They also have ability to ingest blue-green algae (Seale and Beckvar, 1980). Ruibal and Thomas (1988) have published that the larvae of *Lepidobatrachus laevis* feed on prey of their own size and only eat prey that moves or is moved. Larvae of *Dendrobates histrionicus* eat unfertilized eggs of the mother (Zimmermann and Zimmermann, 1982; 1985). Tadpoles of *Scutiger sikkimensis* were predaceous rather than herbivorous (Liu, 1943). Tadpoles of *Caratophrys ornata* are largely cannibalistic, feeding on larvae of other frogs (Noble, 1931). In a mixed culture, tadpoles of *Rana tigerina* predate over *Bufo melanostictus* larvae (Hota and Dash, 1983). Wilbur (1977a) and Dash and Hota (1980) described tadpoles as omnivorous.
Regardless of larval feeding habits, all anuran adults are carnivorous. With metamorphosis in most anuran species, feeding mechanisms shift to prey capture by tongue extrusion (Duellman and Trueb, 1986). As a consequence of different diets, herbivorous anuran larvae and their carnivorous adults differ in the structural/functional nature of their digestive systems. The greatest dedication to herbivory occurs in the digestive system of anuran larvae. In summary, the ontogenetic transition in feeding is from herbivory (anuran larvae) or carnivory (Urodele and Caecilian larvae) to carnivory (adults of all amphibians). The morphological, physiological and biochemical makeup of the digestive system of amphibian larvae reflects the transition. Within the broad categories of herbivory or carnivory, however, is a wide variety of diets, ranging from algae to aquatic plants in herbivorous larvae, to crabs (Rana cancrivora) and rodents in carnivorous species.

1.5 Reproductive Biology:

Animal populations change over time. It is the reproductive ability that leads to the continuity of the organisms of a population. The reproductive potential is always considerably greater than that actually achieved (Southwick, 1972). The environmental factors such as rainfall, humidity, light and temperature have profound effect on the breeding activity (Savage, 1961) which is associated with different types of sexual behaviours. In tropical and subtropical climates temperature is rarely a limiting factor and breeding in most species is closely tied to rainfall. Where there are definite rainy and dry seasons, the breeding season for most forms coincides with the rainy season. In regions where rain occurs in all
months breeding is typically non-seasonal with gametogenesis and spawning occurring in all months of the year (Ingcr and Greenberg, 1963; Zeller, 1960). Einem and Ober (1956) found rainfall to be the most readily identifiable factor associated with breeding in a number of Floridian species. Storm (1960) observed that breeding in *Rana aurora* was most stimulated by relatively high temperatures together with rain. Similarly Heusser (1960) found that both rainfall and temperature acted to compliment a time-dependent migrating drive in the initiation of migration to the breeding pond in *Bufo bufo*.

The use of auditory cues in breeding orientation in amphibians appears to be limited to the Anura. The breeding call in most species is limited to the male, which typically calls in chorus with other males. The possibility that calls may serve to direct the movement of both males and females to the breeding site has long been recognized (Storer, 1925; Noble, 1931). Tracy and Dole (1969) found that *Bufo boreas* moved towards an artificial chorus at night. Movement to the breeding site without a chorus has been observed in *Rana temporaria* (Savage, 1961) and in *Bufo bufo* (Heusser, 1964). Olfaction may direct the breeding migrations in both anurans and salamanders (Savage, 1961; Dole 1968). Blinded cricket frogs retain the ability to utilize a sun compass for migration (Taylor and Ferguson, 1970). Hubbs and Martin (1967) noted in *Bufo valliceps* that, the increased humidity in the area of the pools was the primary attraction for breeding migrations.

In anurans, mating occurs while the male clasps the female with his arms in either Pelvic or Pectoral embrace, or some variant
thereof. Rabb and Rabb (1963) have suggested that the pectoral clasp is adaptively superior as it allows closer juxtaposition of cloacae, thereby promoting more efficient fertilization. The duration of amplexus may differ greatly between species of anurans, the longest amplexus known is that of *Atelopus cruciger* (Sexton, 1958). In most forms, however, amplexus lasts no longer than a single night, and in many species mating is completed within a couple of hours. Differences in total time depend on variation in preovipositional clasping time, time involved in oviposition, and time until release following completion of spawning time required for oviposition is related in a general way to the mode of deposition of the eggs and the number of eggs involved. In *Pelobates fuscus* where the eggs are deposited in one or a few masses (Eibl-Eibesfeldt, 1956) oviposition may be completed within a matter of minutes. *Hymenochirus boettgeri* deposits a large number of eggs in many small groups at the surface of the water, the period of oviposition may last as long as seven hours (Rabb and Rabb, 1963).

Dole and Durant (1974) observed the courtship behaviour associated with calling of *Colostethus collaris*. Both male and female *Eleutherodactylus coqui* give aggressive calls while defending retreat sites (Stewart and Rand, 1991). Kanamadi, Schenider, Hiremath and Jirankali (1993b) studied the vocalization of the tree frog *Polypedates maculatus*, the advertisement calls of two anuran amphibians *Rana tigerina* and *Tomoptera breviceps* by Kanamadi Hiremath and Schneider (1993a). Salthe and Mecham (1974) have made detail investigations on reproductive and courtship patterns of amphibians which cover the reproductive cycles and breeding seasons, breeding sites, factors associated with
mating and species recognition, breeding behaviours and oviposition, ovum size, reproductive patterns, parental care and viviparity, adaptations of egg stages, larval adaptation and ecological and life history aspects of metamorphosis. The mating habits of toad *Bufo bufo* have been studied by (Davies and Halliday, 1977; Gittins et al., 1980) and of the green frog *Rana clamitans* by Wells (1977).

Mohanty-Hejmadi (1974c), Daniel (1975), Dutta and Mohanty-Hejmadi (1976), Mohanty-Hejmadi and Dutta (1978), Dash and Hota (1980), Mishra and Dash (1984), and Mahapatro and Dash (1990) reported the breeding habits of Indian amphibians. Mohanty (1984) has reported the induced breeding of Indian frogs, while Rugh (1934) reported it on the other exotic frogs. Literature on the breeding habits of exotic amphibians include the works of Sever and Dineen, 1978; Collins and Wilbur, 1979; Balinsky, 1985; Solano Haydee, 1987. In general the production (reproduction) efficiency of an animal depends primarily on the efficiency of three basic functions: (i) Male-Female ratio (ii) Reproductive potentiality of females and (iii) Survivability and growth of the young to reach adult stage (Dickerson, 1970). In the present study, more stress have been given in the growth and survivability of the microhylid *Microhyla ornata* than the adult biology.

The life-history of the two major groups of amphibia, Urodela and anurans, is generally characterised by early free-living larvae adapted to an aquatic environment and adult stages adapted to diverse terrestrial habitat (Etkin, 1969). The transition period between these two ecologically distinct phases occurs during the period of metamorphosis in which the free living aquatic larvae
transform into amphibious and terrestrial adults. Previous studies (Taylor and Kollros, 1946; Etkin, 1964) have shown that the nature of this transformation varies considerably by the degree of specialisation of each phase for its own ecological niche. The larval phase is of much importance to the overall life history of frogs and toads (Turner, 1962).

The amphibians have long been noted for their developmental flexibility with various developmental events of the life history, particularly metamorphosis and sexual maturity occurring in different sequences. Development is a continuous process but in order to indicate how far it progressed at any particular time in a species, it is necessary to divide its entire developmental period into series of discrete, readily distinguishable stages, usually arranged in tabular form. Such stagnation is important for comparative studies of development between different species, between individuals of the same species and also for critical evaluation and comparison of results obtained by different workers. The most useful criteria for stagnation are easily recognisable external features because they facilitate identification of specific stages for investigations on living as well as dead individuals of the species concerned.

Sexton and Bizer (1978) have divided the life cycle of an amphibia in four distinct stages such as, (1) the egg (2) the larvae (3) the non-reproductive sub-adult and (4) the reproductive adult. In general, the first two stages are aquatic, while the third and fourth stages are mainly terrestrial in nature. Due to the sequential changes, many of the workers have divided the larval life period into a number of stages, which are represented in the form of normal
tables. Gosner (1960) proposed a generalized series of 46 stages which is broadly applicable to any anuran species having an aquatic larval phase in its life cycle. However, different species vary from each other in several respects such as duration of embryonic and larval period, time intervals between successive stages, sequence and speed of development of certain recognisable features and suitability or otherwise of specific characters for stage recognition etc. Rugh (1962) reproduced several of these tables in his manual of experimental embryology. Khan (1965) has compared the normal tables and relevant data for several anuran species and pointed out these variations. Such differences are also found among various urodelan species (Schrekenberg and Jacobson, 1975). Therefore, the generalized series proposed by Gosner does not exclude the need for accurate stagnation of the developmental period in individual species. In fact, Rugh (1962) has emphasised the need for preparation of normal tables of many amphibian species not only from the point of view of their usefulness for research but also to determine the extent of deviations from the standard pattern of development in this group.

Several developmental biologists have worked out the normal table of different amphibian larvae. Some of the works in this line are *Rana pipiens* (Taylor and Kollors, 1946); *Rana temporaria* (Moser, 1950), *R. fusca* (Kopsch, 1952). Cambar and Marrot (1954) have divided the total larval period, from two cell stage till the resorption of tail, into 54 number of stages in case of *Rana dalmatina*. Few other works on *Rana* species are *R. cyanophlyctis* (Ramaswami and Lakshman, 1959); *Rana tigerina* (Agrawal and Niazi, 1977). Staging of the developmental period of *Bufo* species
have been worked out by Delconte and Sirlin (1952) for *Bufo arenarum*, Rossi (1958) for *Bufo bufo*, Sedra and Michael (1961) for *B. regularis*, Khan (1965) for *B. stomaticus* and Shivpal and Niazi (1979) for *B. andersoni*. Similar studies in other anuran species are Eakin (1946) for *Hyla regilla*, Gallien and Houillon (1951) for *Discoglossus pictus*, Nieuwkoop and Faber (1956) for *Xenopus laevis*, Mishra (1984) for *Rhacophorus maculatus*, Mahapatro (1986) for *Bufo stomaticus*.

**Physiological and Ecological Aspects of Reproductive Biology:**

(a) **Reproductive cycle and breeding season**:

The breeding season in anurans include those times during which chorusing by males, as well as spawning may occur. It has been repeatedly observed that the threshold for reproductive behaviour is much lower in males than in females, and the "calling season" for most species is considerably longer than the true breeding (spawning) season. Males usually arrive first at the breeding site, generally remain there longer, and may call many times when conditions don't favour spawning. A male may also mate more than once during any particular season. Females of most species, however, spawn only once a year, and a few forms of more northern temperate climates, such as *Rana pretiosa* (Turner, 1960) or some species of *Bufo* (Blair, 1943) do not breed every year. Some more southern species like *Bufo valliceps* may deposit more than one set of eggs per season, and many tropical species with low egg numbers probably spawn more than once a year (Turner, 1962).
The onset of breeding in most temperate anuran species is roughly associated with the increasing environmental temperatures from early spring to summer, although the date of onset and duration of the breeding season varies between species from year to year. A few forms like *Rana pipiens berlandieri* and *Pseudacris clarkii* in Texas show a tendency towards two breeding seasons, with spring and autumn breeding peaks.

In some temperate zone species breeding takes place following heavy rains at any time during the warmer months which are adapted to spawning in temporary ponds formed by infrequent rain storms. Some species like *Scaphiopus h. holbrookii* in regions of high rainfall may lack definite breeding season and breed following heavy rains in almost any month of the year (Gosner and Black, 1955). In tropical and subtropical climates, temperature is rarely a limiting factor and breeding in most species is closely tied to rainfall. Where there are definite rains and dry seasons, the breeding season for most forms coincides with the rainy seasons. The species that breed during dry periods spend their entire life in uniform microhabitats (Alcala, 1962). In regions where rain occurs in all months breeding is typically non-seasonal with gametogenesis and spawning occurring in all months of the year (Inger and Greenberg, 1963; Zeller, 1960).

b. **Extrinsic Factors**:  

Extrinsic factors that have been proposed as influencing reproductive cycles in anurans include food, light, rainfall and temperature. Adequate nutrition is essential to reproduction, particularly in females where the accumulation of lipid reserves are
necessary for vitellogenesis. Rabb and Rabb (1963) observed that light does influence reproductive activity over short periods in some forms including *Hymenochirus*. Seasonal variations in rainfall clearly influence breeding activity in many anurans, the fact that male secondary sex characters may regress during the dry season in many tropical species (Noble, 1931). In *Bufo regularis* in Congo, the development of male breeding characters actually anticipates the onset of the rainy season and regresses before the end of the rainy season (Inger and Greenberg, 1956). This clearly suggests a genetically conditioned cycle regulated by seasonal rainfall.

There is a direct relationship between temperature and the amphibian spermatogenic cycles. The spermatogenic cycle is essentially continuous in tropical and subtropical forms, but is interrupted or impaired during the autumn and winter in most species of temperate regions. The impairment in many forms is tied directly to lower autumn and winter temperatures that affects the gonadotropin secretion of the pituitary and sensitivity of the germinal epithelium to gonadotropic hormone. Exception to this is *Rana temporaria* in which form the germinal epithelium enters into a resting period that begins in the late summer and extends through the winter. Not only is the resting period initiated when environmental temperatures are still high, but the germinal epithelium remains relatively insensitive to high temperatures and gonadotropin during the first part of the resting period. This indicates that the spermatogenic cycle in this species is independent of environmental factors and depends on an internal genetically determined rhythm.
c. Factors Associated with Initiation of Breeding Activity:

Rainfall is the most obvious factor in the stimulation of breeding especially in species that utilise more temporary sites. This is found to be true for most species of the Transvall highveld, although reactivity to rain generally declined later in the breeding season. *Bufo regularis* is a species that confines its spawning to permanent streams and ponds in this region, showed no response to rainfall. Einem and Ober (1956) found rainfall to be the most readily identifiable factor associated with breeding in a number of Floridian species. Spawning in *Rana aurora* is associated with the attainment of a minimum water temperature of about 7°C. Storm (1960) observed that movement to the breeding pond in *Rana aurora* was most stimulated by relatively high temperatures together with rain. Heusser (1960) found that both rainfall and temperature acted to compliment a time-dependent migrating drive in the initiation of migration to the breeding pond in *Bufo bufo*.

d. Location of Breeding site and Formation of Breeding Aggregations:

Amphibians are divisible into two incompletely delimited groups with respect to the degree of movement associated with breeding. In terrestrial breeding species such as plethodontid Salamanders (Gordon, 1952) or certain leptodactylid frogs (Goin, 1947), mating and oviposition tend to take place within or close to the home range or area "of familiarity" of the individual. The second series includes species in which the adult normally resides some distance from water, but breeding depends on water; that is,
eggs are deposited on or near the water and there is an aquatic larva. Breeding, therefore, may depend on a major movement to the breeding site. Green frogs (*Rana clamitans*) may aggregate in specific areas in marshes during the breeding season, with some individuals moving as far as 500 yards (Oldham, 1967).

The breeding call in most species is limited to the males, which typically call in chorus with other males. Call may serve to direct the movement of both males and females to the breeding site (Storer, 1925; Noble, 1931). Breeding call is absent in *Bufo boreas* (Tracy and Dole, 1969) but it moved towards an artificial chorus at night but oriented in the direction of their migratory movement when the sun was visible. Although it is clear that call may play a role in many anurans in directing movements to the breeding site, it is equally clear that other factors may predominate. Movement to the breeding site without a chorus has been observed in *Rana temporaria* (Savage, 1961) and *Bufo bufo* (Heusser, 1958, 1964). Movements towards a breeding site in the weakly calling *Bufo bufo* have been observed at distances beyond the audible range of a chorus (Moore, 1954).

Olfaction may direct breeding migration in both anurans and salamanders. Savage (1961) argued that olfaction is the critical sense used in migration of *Rana temporaria* to the breeding ponds. Oldham (1967) demonstrated that the homing response with respect to breeding site in *Rana clamitans* was significantly reduced with ablation of the olfactory sense. Tracy and Dole (1969) found that anosmatic *Bufo boreas* were severely disoriented at night when displaced 50-200 m from the breeding site. These workers concluded
that olfaction was the most important sense involved in nocturnal homing behaviour of these species. Odor plays an important, though not exclusive, role in orientation of some species. Odor of the receptive female in Pipa pipa appears to assist in the attraction and stimulation of males (Rabb and Rabb, 1963), and secretions of the postaxillary glands of the male Hymenochirus possibly repel other males or attract females (Rabb and Rabb, 1963).

Visual factors are also important in amphibian migrations. The amphibians orient to a particular compass direction when provided with a celestial cue such as the sun, moon and stars. The most commonly used celestial cue appears to be the sun, but some ability to orient to star and the moon has been indicated for cricket frogs, Acris and blinded cricket frogs retain the ability to utilize a sun compass (Taylor and Ferguson, 1970).

New sites may attract breeding congresses within a short period in many species like Bufo valliceps. Some animals may simply move to the site which offers strongest immediate attraction in terms of some critical sensory cue. On the other hand, individuals of some species have a strong attachment to a particular site or even to a particular spot at the breeding site during the breeding season and even from season to season (Oldham, 1967; Heusser, 1960). Heusser (1960) observed that Bufo bufo returned for two consecutive years to the site of a pond that had been covered by a newly constructed road.

A wide range of sensory cues may be involved in breeding movements to a breeding site. In view of the different types of possible cues, variations may be expected between species and even
within the same species under different conditions. The varying degrees of attachment to a particular site, and the totality of factors and their relative roles in any one case may be exceedingly complex and difficult to unravel.

**e. Role of Vocalization in Reproductive Biology:**

Vocalization is one of the most distinctive features of frogs and toads. Calls play a profound role in their reproductive biology. Both sexes in anurans may emit sounds, but males are generally much more vocal, and mating calls are restricted usually to that sex. The ability to produce calls associated with breeding tends to be seasonally limited in cyclic breeders, and is influenced by hormones. Mating calls undoubtedly have social significance in terms of behavioural and physiological facilitation of breeding in many species. Calling by less aggressive males in many chorusing aggregations is obviously stimulated by the calls of more active individuals, leading to a more intense chorus that would be more effective in attracting females. At the same time, many males that are stimulated to call by other males would obtain mates as a direct result. It is also probable that chorusing triggers neuro-endocrinological changes in ripe females of some species that stimulates the mating response and may also influence ovulation.

**1.6 Ecological Studies on Growth and Metamorphosis:**

Hammet (1936) defined growth to include development as "growth is the coordinated expression of incremental developmental factors". Ecologically growth may be defined as the biomass change
over time of an individual and the biomass is measured as live weight, dry weight, protein weight, calories etc. Growth in the sense of increase in lean body mass begins in early embryonic life and may continue into adulthood in amphibians. This is restricted to post-metamorphic growth. Growth and metamorphosis play vital role in the life history of amphibians and in animals, who undergo two different life stages. In anuran larvae growth is essential for metamorphosis which on the other hand is required for the continuity of generation. Metamorphosis is referred to the sequential drastic post-embryonic development in the non-reproductive structure of an organism in a stipulated time period and it transforms the non-reproductive one to an individual that can reproduce. In other words as Bennett and Frieden (1962) put it "metamorphosis is a developmental transition from a larval form specialised for a particular existence to an adult form adapted for another environment. Therefore, at metamorphosis amphibians acquire their final proportions for which post-metamorphic growth is approximately isometric.

Savage (1935 a, b, 1937 and 1952) initiated ecological studies of anuran tadpoles but it was restricted to the general natural history of the organism. Other studies have dealt with growth rates, food habits, tadpole predators, and other aspects of the natural history of various anuran species (Gosner and Black, 1955 - growth rate; Jenssen, 1967; Sekar 1992 - food habits; Anderson, 1968 and Werner and Anholt, 1996 - predators). Herried and Kinney (1966) studied the survivorship of Rana sylvatica and estimated the mean mortality rate to be 96%. Brockelman (1969) studied the growth and survivorship of a population of Bufo americanus tadpoles. Calef
(1973) integrated these approaches to the natural condition and studied the natural mortality of a population of *Rana aurora* tadpoles.

Growth rate of each stage is an important part of the life history strategy of a species (Gadgil and Bossert, 1970) and growth rates of individuals from different populations may vary markedly (Licht, 1975). Body size is influenced by varying degrees of environmental conditions. In poikilothermic animals it is apparent that body size is equally controlled by many conditions.

It has been found out by various workers that certain environmental factors, as mentioned below, stimulated the metamorphic process.


3. **Predation**: Skelly, 1992; Guscon, 1995; Anholt and Werner, 1995; Werner and Anholt, 1996.


Besides the above mentioned factors there are some other environmental factors which have a great influence on the growth and metamorphosis of the larvae such as available of active space, population density, availability and quality of food, disease etc. Hence, it is generally argued that the physiological response of metamorphosis is somehow coupled with environmental parameters which predicts advantages for becoming terrestrial (Wilbur, 1972; Wilbur and Collins, 1973).

The review of literature states that the anuran larvae have been subjected to many ecological studies. Crowding studies or density stress on anuran larvae has been shown to have detrimental effects on the growth, survivorship and time of metamorphosis (Richards, 1958 and 1962; Rose 1960; Licht, 1967; Brockelman,
1969; Wilbur, 1972, 1976, 1977a,b; Gromko et al., 1973; Wilbur and Collins, 1973; John and Fenster, 1975; Smith-Gill and Gill, 1978; Smith-Gill and Berven, 1979; Dash and Hota, 1980; Wilbur, 1980; Semlitsch and Caldwell, 1982; Smith, 1983; Mishra and Dash, 1984; Petranka and Sih, 1986; Mahapatro and Dash, 1987; Petranka, 1989; and Scott, 1994). These characteristics are presumably important in interpreting the adaptive nature of complex life cycle of anurans (Wilbur, 1980).

The relationship between the body size at metamorphosis and the length of larval period has been studied by many workers. Collins (1979) found that size at metamorphosis may vary widely or vary little (Travis, 1981) within a larval population. According to Smith (1987) frogs that metamorphosed at large size maintained their size advantage at maturity. Large body size and early date at metamorphosis increased survivorship to maturity. There is a positive correlation between these two parameters (Wilbur, 1971; Shoop, 1974; Travis, 1980 and 1984). Optimal sizes are independent of the time of metamorphosis (Rowe and Ludwig, 1991). Therefore the positive correlation is not consistent among populations of a species (Collins, 1979), among distinct full sibling groups drawn from one population and reared in the laboratory (Travis, 1980) or among sub-samples of distinct full sibling groups reared under different environmental conditions (Travis, 1983). Metamorphic size and length of larval period are important in amphibians because of their influence on larval and juvenile survival and the potential level of adult fecundity (Tevis, 1966; Wilbur, 1971 and 1972; Shoop, 1974; Collins, 1975 and 1979; Smith-Gill and Gill, 1978; Berven et al., 1979; Travis, 1981; Dash and Hota, 1980 and Berven 1982).
Though the food and food habit of tadpoles have been studied extensively, yet the search for a suitable food for the successful laboratory culture of anuran larvae continues. In the present study, stress is given to quantify the effect of food quality on the growth rate, body size at metamorphosis and the length of the larval period of *Microhyla ornata* larvae in laboratory conditions.

Environmental conditions such as food availability and larval density directly or indirectly affected the growth and metamorphosis of anuran tadpoles. Riha and Berven (1991) found in *Rana sylvatica* that developmental time was negatively correlated with population density in some environment and in the other, population density had no effect on developmental time but according to Newman (1994) tadpoles from both increasing density and decreasing food treatments metamorphosed earlier than tadpoles from constant density and constant food treatments. Larvae in ponds grew faster, had shorter larval period and were larger at metamorphosis than stream growing larvae. Competition appears to be important in the ecology of tadpoles of many anuran species. Intraspecific competition can have major effects in both the field (Wilbur, 1972 and 1976; Morin, 1983; Smith, 1983 and 1990) and in the laboratory. Intraspecific competition can be caused by exploitation (the depletion of resources), or by interference. In interference competition some tadpoles either reduce the access of others to preferred resource (Savage, 1952 and Steinwascher, 1978 a, b), increase levels of stress (Adolph, 1931; Gromoko *et al.*, 1973; John and Fenster, 1975; Mishra and Dash, 1984 and Mahapatro and Dash, 1987) or release of allelopathic chemicals or symbiotes (Richards, 1958; Rose, 1959, 1960; Licht, 1967). Similarly, competition can be
caused by fouling of the water in laboratory experiments (Wilbur, 1977b; Dash and Mahapatro, 1990 and Murray, 1990). Petranka (1984) showed that allelopathic effects can occur in natural ponds when animals are present at high densities. The tadpoles responded strongly to density but the effects of interference and exploitation could not be separated (Dash and Hota, 1980; Sokal, 1984). Petranka (1984) found that growth occurred independently of density when larvae were given identical per capita food allotments at low levels or were fed *ad libitum*. Newman (1994) also observed that lower food levels decreased the larval growth and body mass at metamorphosis but the average larval time varied to reach that size.

Breden and Kelly (1982) found that increased conspecific interaction among tadpoles of *Bufo americanus* has affected the body mass at metamorphosis and the time for metamorphosis. Savage (1935 a,b, 1937, 1939, 1952 and 1961) studies the life history and ecology of young larvae of European frogs and toads with regard to the adaptations to the habitat of mass spawning, distribution of spawn ponds, nutrition and their physiology and anatomical observations. Species distributions and occurrence patterns can be determined by biotic factors such as predation (Kettlewell, 1955; Werner *et al.*, 1983; Watanabe, 1984; Resetarits and Wilbur, 1989; Woodman and Price 1992) and competition (Grosberg, 1981; Hairston, 1986; Robertson and Gaines 1986; Alatalo *et al.*, 1987) and by abiotic factors (Kingsolver, 1989; Dunson and Travis, 1991). However, other studies have emphasized the importance of predators in preventing competition for food (Herried and Kinney, 1966; Calef, 1973 and Wilbur *et al.*, 1983).
which also directly affect the larval survival by eliminating the anuran larvae (Smith, 1983).

Size of an anuran larva at metamorphosis and timing of metamorphosis during the growing seasons are important life history characters because they affect body size and age at first reproduction (Collins, 1975), while the successful metamorphosis of the tadpoles regulate the abundance of juvenile frogs and toads and hence the adults. Size at metamorphosis may affect juvenile physiology or performance (Pough and Kamel, 1984; Taigen and Pough, 1955; John-Alder and Morin, 1990; Newman and Dunham, 1994), survivorship (Martof, 1956; Pfenning et al., 1991); and size, age, and reproductive success at maturity (Turner, 1962; Clarke, 1974; Berven, 1982; Smith 1987; Semlitsh et al., 1988; Berven, 1990); Although a lot is known about the underlying physiological mechanisms and biochemical regulation of metamorphosis, no adequate predictive ecological theory of metamorphosis exists. The models and discussions presented by Wilbur and Collins (1973) and Collins (1979) provide the much needed beginning of the synthesis of many aspects of amphibian metamorphosis. The model (Fig. 1.6.1) assumes that metamorphosis only takes place over a limited range of body size. The lower limit must be achieved by a larva before metamorphosis and metamorphosis occurs when the upper limit of body size is reached regardless of environmental conditions. But the upper size is realized only under optimum environmental conditions. Also, this model assumes that size specific growth rate is the mechanism through which a larva assesses environmental quality.
1.7 Physiological Aspects of Growth and Metamorphosis:

All vertebrates undergo complex morphological, physiological, and biochemical transition as they develop from a single fertilized cell into the terminal, sexually mature or "adult" form of their species. The term metamorphosis is used for any and all morphological, biochemical, and physiological, non-reoccurring changes that occur during the entire larval climax stages.

In anuran larvae, the growth and differentiation of the hind limbs usually define the earliest stages. The forelimbs may or may not be enclosed in the branchial chambers; if they are enclosed, the forelimbs erupt in late larval stages. During the final stages tail regresses and the adult mouth forms.

Most adult amphibians are terrestrial, yet their larvae are aquatic. Thus two life stages clearly experience different environments. The many changes in both the internal and external environments that amphibians face during their life span require changes in the integrative capacities of the animals. The two major systems namely the nervous and endocrine systems involved in integration and control change greatly during metamorphosis.

Growth-Promoting Hormones:

Autotransplantation of the pituitary leads to gigantism of larvae, while hypophysectomized animals are smaller than normal (Etkin and Lehrer, 1960) indicating that the larval pituitary contains
a growth-promoting hormone under inhibitory hypothalamic control. Herman et al. (1964), Dodd and Dodd (1976) showed that prolactin can act as a growth factor in amphibian larvae. Aside from prolactin effect on amphibian larval growth, it also inhibits metamorphosis at the peripheral tissue level (Bern et al., 1967, Etkin and Gona, 1967). On the other hand Etkin (1968) and Bern et al., (1967) suggested a major role of prolactin during metamorphosis and also predicted changes in circulating prolactin levels. Prolactin levels should be high during early larval stages, causing larval growth, and should decrease before climax and then drop to very low levels during climax which would allow thyroid hormones to cause the many reported effects on tissues during climax (Frieden and Just, 1970). This hypothesis rested on prolactin's antimetamorphic effects, its growth-promoting capabilities, and the development of the medium eminence just before climax, when it presumably could send an inhibitory signal to suppress prolactin release. This hypothesis appeared to find early support when bioassays found more prolactin in the pituitary of young salamanders (Norris, Jones and Cohen, 1973) and bull frog larvae (Hsu, Yu and Liang, 1976) than in the pituitary of climax larvae of the same species. The above hypothesis is not again correct as the measurement of prolactin by RIA soon provided evidence that the pituitary and serum prolactin levels are low during early stages of development and increase at climax stages (Yamamoto and Kikuyama, 1982; Yamamoto, Nihuma and Kikuyama, 1986). Again prolactin receptor number is under hormonal control. Thyroid hormones increase prolactin receptor number in all tissues (Carr, Jacobs and Jaffe, 1981). The number of prolactin receptors in various tissues is highest right before or at
climax (Carr, Jacobs and Jaffe, 1981), but receptor numbers are lower in young larvae and at late climax stage.

Unfortunately, no data exist on prolactin receptors in either the thyroid or pituitary gland during amphibian metamorphosis.

Adrenal steroids apparently accelerate metamorphosis only when the circulating levels of thyroid hormone are high (Kikuyama et al., 1983). Estrogen may also accelerate thyroid hormone-induced metamorphosis (Frieden and Naile, 1955), but the sex steroids progesterone, estrogen and testosterone are not required to accelerate metamorphosis (Kikuyama et al., 1983). All authors agree that the lowest circulation levels of steroid hormones occur in early limb bud stages and increase during rapid hind limb growth. Dale (1962) first attempted to measure the steroid hormone production during the entire metamorphic period and found that *Rana pipiens* and *R. sylvatica* excreted hydrocortisone at very early limb bud stages, whereas *Xenopus laevis* does not excrete the first corticoid hormone until mid larval stages (Leist, 1970).

Etkin (1968) and Kollros (1961) suggested that for normal metamorphosis the concentration of thyroid hormones must increase continuously in the blood of larvae. This hypothesis resulted from studies of thyroidectomized and/or of hypophysectomized larvae that were induced to undergo normal metamorphosis by immersion in increasing concentrations of thyroid hormones. This hypothesis was proved by Just (1967, 1972) who measured the increased blood thyroid hormone levels during larval development in *R. pipiens* and
the same level decreased when the thyroid gland was removed. All authors (Regard, Taurog and Nakashima, 1978; Suzuki and Suzuki, 1981) agree that the levels of hormones are low in early larval stages, increase dramatically during climax, then drop precipitously after tail loss and remain low in the adult but the major disagreement lies in the timing of maximum increase and the absolute amount of hormones released.

The following figure illustrates role of thyroid gland in amphibian metamorphosis:

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Development of median eminence

↑

Tetraiodothyronine ← THYROID HORMONES → Peripheral thyroid hormonal receptor
diodonation

↓

[(The hormone stimulates (filled arrows) certain events while inhibiting (open arrows) others]
A proposed model explaining the role of hypothalamus in the control of metamorphosis. The model accounts for the need of the hypothalamus as well as the inability of the thyrotropin-releasing hormone (TRH) to induce climax stages. Corticotropin-releasing hormone (CRH) and gonadotropin-releasing hormone (GnRH) can induce thyroid-stimulating hormone (TRH) before climax and that steroid hormone induce pituitary TRH receptors, follicle-stimulation hormone (FSH) and luteinizing hormone (LH).
Predictor of Transformation Time:

Developmental biologists typically predict climax times of amphibians based on differentiation rates rather than larval size. Wilbur and Collins (1973), Wilbur (1976), Dash and Hota (1980), Hota and Dash (1981), Mishra and Dash (1984), Mohanty and Dash (1986), Mahapatro and Dash (1987), Dei et al. (1994) have found that body size and growth rate are most important in predicting transformation times. They suggest that larval transformation cannot occur at least than a certain minimum size. However, no correlation has been established between body size of the larva and thyroid development. But there may be a distinct possibility to suggest that a minimum and/or maximum larval size is necessary for thyroid to develop and secrete enough hormones to induce metamorphic climax. Smith-Gill and Berven (1979) showed that 95% of the variance in the length of the larval life can be accounted for by differentiation rates, while body growth rates account for less than 50% of the variance. These findings may quantitatively confirm the subjective observations of developmental biologists who view stages as the predictor of metamorphic climax. But larval stages are related to growth rates which could indirectly trigger endocrinological changes that may alter transformation rates (Wassersug, 1986). Growth rates depend upon nutrition level and other factors. It has been suggested that larvae excrete prostaglandins from their oral mucosa when feeding, and that these compounds retard metamorphosis and when food is scarce, less mucus and prostaglandins are produced causing metamorphosis. But the above theory may not be correct as prostaglandin administration
does not affect metamorphic rate (Mobbs, King and Wassersug, 1988).

If food available to larvae changes, the secretion rate of any of the many gastrointestinal hormones, it may affect the pituitary-hypothalamus axis to release the pituitary hormones causing metamorphosis. This type of feed back would help integrate the ecological and endocrinological view of the control of metamorphosis, but it is not known whether food in the gastrointestinal tract affects the hormone levels. Cholecystokinin appears just after hatching and attains its highest levels in mid larval stages, then declines during early climax (Scalise and Vigna, 1988). Iodine is essential for functioning of thyroxine and can alone initiate metamorphosis.

More study is required to correlate ecological aspects of larval growth, food availability and nutrition level and other ecological factors with growth of endocrine system and hormone secretion so that an integrated ecological-endocrinal control mechanism of amphibian metamorphosis is fully understood. Triggering of metamorphosis may be an environmental signal affecting the larval brain through nervous system or endogenous clock in the hypothalamus.

1.8 Energetics:

Life is defined physico-chemically as “an organized unit in an open thermodynamic system with continuous influx and outflux of matter and energy maintained in a steady state, with the properties of specific biosynthesis and homeostasis” (Das, 1978). Hence,
energy plays a vital role in the life of living organisms. The manner in which an animal manipulates its energy, consumption and expenditure is important for its growth, reproductive success and survival (Merker and Nagy, 1984). An animal must trade off the benefits of its actions (energy or nutrients acquired) against the costs (time and energy lost from other activities, exposure to predators, disease, and environmental stress (Krebs and McCleery 1984; Pyke, 1984; Schoener, 1987). An organism's life-history may be viewed upon as resultant of three biological processes such as maintenance, growth and reproduction (Gadgil and Bossert, 1970). Organisms are considered as resource transformers which partition a finite input of food or energy into these biological processes, so it should be straightforward to appreciate this aspect of their physiology in ecological terms. Such an approach has its root in ecological energetics (Mac Arthur, 1962; Phillipson, 1966). To quote Gates (1975) "climate is coupled to an animal through flow of energy. By understanding the complete energy budget of an animal, productive generalization can be produced about life processes which are directly linked to the environment". Also, precise data on energy budget of individual organism are basic to the understanding of an ecosystem energetics (Macfadyen, 1963; Darnell, 1968; Dash, 1985). Such estimates used in conjunction with the life tables of organisms would provide a correct method of analysing the relationships that exist between life processes and dependent environment.

Anurans have complex life cycles featuring a dramatic shift in habitat which includes an abrupt ontogenetic change in an individual's morphology, physiology and behaviour. They have
developing aquatic larva that eventually metamorphoses into a small version of the terrestrial adult, morphologically quite different from the aquatic larva (Just et al., 1981; Wassersug, 1984). The larval and adult anurans are ecologically independent so as not to compete for the same resources. Such complex life cycle forms are often viewed as adaptations to exploit transient opportunities for growth and dispersal (Wilbur, 1984). Thus, it implies that larval anurans must metamorphose into juveniles so that they can migrate to new habitats to facilitate breeding.

The adaptive significance of the larval stage of anurans is believed to reside in their ability to exploit transient opportunities for rapid growth in temporary ponds. The growth opportunity, together with the mortality risk influences the timing of anuran metamorphosis i.e. a shift in the utilization of the resources of different habitat (Werner, 1996). As ectotherms, amphibians are well adapted for low energy flow and sufficient biomass production (Pough, 1980). They are more sedentary than endotherms and do not use metabolic energy to raise body temperatures. Larval anurans are referred as the “energy gathering stage” (Salthe and Mecham, 1974). Crump (1981) suggested that energy accumulation may be an important determinant in determining the timing of anuran metamorphosis. The feeding rates of larval anuran interacting with a variety of environmental factors, is a good quantitative predictor of growth and development rates and hence energy content at the time of metamorphosis and larval duration (Pandian and Marian, 1985a). Greater energy accumulation at the terminal stages of larval anuran is advantageous for the organism as it enhances metamorphic efficiency (Pandian and Marian, 1985b). According to Seale (1987),
the physiological, ecological and biochemical components of amphibian energetics are complex and incompletely understood. The adapted complex life cycle pattern of anurans to exploit aquatic and terrestrial food resources increases the energy resource base of the organisms. The foraging activity of the terrestrial adult anurans influences the energetic parameters of their aquatic larva (Dash, 1991).

Many interdependent factors influence the proportion of energy partitioned for maintenance, growth and reproduction in amphibians (Fig. 1.8.1). Amphibians can divert energy from "normal" growth and maintenance to regenerate certain body parts lost from predation or parasitism (Frazer, 1973). Amphibians may be important regulators of nutrient cycling within ecosystems. Furthermore, they may transport material between terrestrial and aquatic ecosystems through migrations and dispersal after metamorphosis. In the "typical" complex life cycle of temperate amphibians, eggs containing energy assimilated by terrestrial adults are deposited into fresh waters where larvae accumulate material and energy and later metamorphose and transport this biomass back to land on dispersal.

Literature review shows that Borutsky (1939) and Lindeman (1942) were among the first ecologists to give the serious and detailed considerations to the identification and measurement of production and energy flow in natural ecosystems. Since then many valuable studies have been reported in this line (Odum, 1957 a,b; Dash and Patra, 1977; Beyers and Odum 1959; Senapati and Dash, 1981, 1983 and Dash, 1985). The process of energy flow in animal
husbandry has been fully described by (Broody, 1945 and Kleiber, 1961). Ivlev (1939 a,b,c) pioneered the studies of energy transformations by fish and aquatic invertebrates. The growth and energy requirements of fish and earthworms have been worked out by a number of scientists (Pandian, 1967; Birkett, 1972; Dash and Patra, 1977; Singh, 1977; Senapati and Dash, 1981, and 1983). Merker and Nagy (1984) have studied the energy utilization by free-ranging *Sceloporus virgatus* lizards. The energetics of various lizards have been studied by many workers (Rose, 1981; Anderson and Karasov, 1981). Bennett (1978) studied the active metabolism of the lower vertebrates. Nagy and Milton (1979), Karasov (1981) studied the energy metabolism of some mammals. Species variation in energy assimilation rate and its allocation to growth and respiration was studied by Konarzewski (1995) in avian postembryonic development. Degani *et al.*, (1989) studied the oxygen consumption of European eel and Liem (1980) studied the energy acquisition in teleosts.

There are a number of references on the energetics of some other animal groups, but works on amphibian energetics are few (Mohanty and Dash, 1988; Dash and Mishra, 1989; and Mahapatro and Dash, 1992). Withers (1980) has determined the oxygen consumption of salamanders during rest, activity and recovery. Cree (1984) compared the rate of oxygen consumption in the tadpoles of two frogs *L. raniformis* and *L. ewingi*. Oxygen consumption and its effect was studied by many workers on adult amphibians as well as on tadpoles (Feder, 1981; 1982; 1983; Wassersug and Feder, 1983; Withers and Hillman, 1983; Mishra and Dash, 1985). Nitrogen excretion in case of amphibians has been studied by many scientists.
(Munro, 1939 and 1953; Withers et al., 1982; Nash and Fankhauser, 1959). Ryan et al., (1983), Mac Nally (1981, 1984) have worked out the reproductive energetics of a neotropical frog and chorusing males (Ranidella, Anura) respectively. Time and energy cost of metamorphosis was studied by Pandian and Marian (1985b, c) in the bullfrog Rana tigerina. Taigen and Wells (1985) discussed the energetics of vocalization by an anuran amphibian. Van Beurden (1980) observed energy metabolism of dormant Australian water holding frogs. The concentration of urea in dehydrated Hyla arborea toads and the urea accumulation as an adaptation to the terrestrial life by Bufo viridis toad have been studied by Degani and Warbug (1984) and Degani et al., (1984) respectively.

Crump (1981) suggested that in addition to growth and differentiation rates, energy accumulation might be important in determining the time of amphibian metamorphosis.

Ecological energy budget for amphibians usually have applied some form of the general equation outlined by Petrusewicz and Mac Fadyen (1970).

\[ C = P + M + F + U \]

Where C is food energy consumed, P is growth (or production), and M, F, and U are losses from metabolism, faeces, and nitrogenous excretory products respectively. In this study, the energy flow equation \(( C = P + R + F + U )\) of Grodzinski et al., (1975) has been followed, where 'C' stands for consumption, 'P' for production, 'R' for respiration, 'F' for egesta and 'U' for excretion.
1.9 Objectives of the Study:

*Microhyla ornata* is a very small sized microhylid frog of this region and considering the little work done on ecological aspects of the larvae and the importance of larval life history on the adult population, this work was designed to study the following ecological aspects:

1. To make a population survey and study the biology of *Microhyla ornata* under field conditions.

2. To study the larval stages and stagnation of the total larval life period on the basis of most distinguishable morphological parameters such as development of limbs.

3. To understand the growth and metamorphosis of tadpoles reared in isolation and in groups.

4. To understand the effect of density on growth and metamorphosis on *Microhyla ornata* larvae.

5. To study the effect of food quality on the rate of growth and metamorphosis.

6. To estimate the energy budget of *Microhyla ornata* larvae in terms of consumption, egestion, assimilation, excretion, maintenance and production and to compare it with other anuran species.
Fig. 1.6.1 Hypothetical control processes determining the stimulation of the neurohormonal process that initiate metamorphosis. Constants \(b\) and \(b+c\) are the species-specific lower and upper limits, respectively, of the body size \(W\) at which metamorphosis will occur. Within these limits, metamorphosis is initiated only if the recent growth is less than the value of \(g\), some function of weight. Recent growth rate \(\frac{dW}{dt}\). Adopted from Wibur and Collins (1973).
Fig. 8.1 The phenotype as a source transformer. Adopted from: Clay and Townsend (1981).