MATERIAL AND GENERAL
EXPERIMENTAL PROCEDURE
The carp *Labeo rohita* is an economically important food fish having great commercial value occurring abundantly in the fresh water tanks in and around Anantapur. Beside its wide availability and commercial importance this carp is known to be suitable test animal to a variety of environmental conditions (Sreenivasan and Swaminathan, 1967; Bashamohidden and Subbarao, 1982; Bashamodidden, 1984).

**1. BIOLOGY OF THE CARP:**

Since this investigation is of an ecophysiological nature a brief account of the biology of the fish is involved in this investigation may provide a good premeable to this study. The carp *Labeo rohita* belong to the exclusively fresh water fishes of the family cyprinidae.

*Labeo Rohita* is the most available major carp found all over northern and Central India upto Godavari river. It is commonly called as rohu, has an elongated body with a moderately rounded abdomen, head prominent with pointed snout, terminal mouth with thicker fringed lips, the body colour brownish grey to blackish above and the scales are with orange to reddish centre. It is a fast growing species. It is cornivorous from feeding on both vegetable and nonvegetable matter.

*Labeo rohita* is one of the most valuable food fish of India, growing very quickly to a length of 45 cm (about
0.7 kg wt.) in the first year and maximally it can also grow up to a length of 91 cm in three years. The Wealth of India, *Fish and Fisheries, 1962*. The carp *Labeo rohita* sexually matures when about two years old. Carp culture has recently become very important in many countries. Hickling (1962) estimated that 700000 tons or 2% of the total world fish production was carp. This type of fish culture represents in the supply of fish protein for human consumption, hence this carp which has economically important food fishes has a great commercial value. By virtue of its omnivorous feeding habits, easy and abundant availability and adaptability to varied environmental conditions and greater commercial value *Labeo rohita* has been selected as the ideal experimental animal for the present investigation.

**GENERAL EXPERIMENTAL PROCEDURES:**

The experimental fish, *Labeo rohita*, weighing 12 ± 1 grams were collected from the local fisheries department, Government of Andhra Pradesh, and stored in large glass aquaria in the laboratory at room temperature (25°C ± 0.5°C) and exposed to natural photo period. A gentle overflow of water was continuously maintained through these aquaria to keep up water renewal therein. The fish were fed daily, and the feeding schedule being frog leg muscle for two days in a week, and ground nut cake and rice bran on the remaining days. Fishes were adapted for a period of two weeks to the laboratory conditions.
The experimental fishes were divided into two batches. One batch of the fishes from the laboratory temperature were adapted to 20°C and another batch of the fishes from laboratory temperature to 30°C in BOD incubator with thermostatically controlled water bath. In either case, the fishes were transferred from laboratory temperature to a lower temperature (20°C) and to a higher temperature (30°C) as the case may be and maintained therein, subsequently over a period of adaptation to lower temperature (20°C), a 12 day sojourn and for adaptation to higher temperature (30°C), a 8-day sojourn, were allowed respectively. It is experimentally verified and confirmed that these periods are sufficient for the completion of adaptations to lower and higher temperature through the estimation of oxygen consumption of the whole fish until the attainment of a constant level in oxygen consumption.

In order to differentiate heat-stress from heat-adaptation, five batches, (each batch consisting of six fishes) of 20°C adapted fishes, were re-adapted very slowly to five different temperature rates i.e. 1°C/24 hrs, 1°C/36 hrs, 1°C/48°C hrs and 1°C/72 hrs from 20°C to 30°C. As the time course experiments with reference to oxygen consumption of the batch of re-adapted fishes subjected to 1°C/60 hrs indicate almost reaching of the controls, and nearly 100% recovery in oxygen consumption over a period of 35 days, hence, the fishes exposed to 1°C/60 hrs from 20°C to 30°C
were taken as "heat-adapted fishes". Simultaneously, similar type of experiments in a reverse way was carried out with the same temperature rates for 30°C adapted fishes to re-adapt to 20°C until the constant values were obtained over a period of 35 days. The fishes exposed to the rates of 1°C/60 hrs also indicated almost 100% recovery. Therefore, the fishes exposed to 1°C/60 hrs from 30°C to 20°C were considered as the "cold-adapted fishes".

In order to induce stress in the fish, the 20°C adapted fishes were re-adapted to an abrupt raise of temperature at the rate of 1°C/hr from 20°C to 30°C. These fishes were taken as "Heat-stressed fishes", whereas the fishes that were re-adapted from 30°C to 20°C with a change in temperature at rate of 1°C/hr are treated as "cold-stressed fishes". Thus the experimental fishes were grouped into four different categories. 1. Heat-adapted fishes subjected to a temperature change from 20°C to 30°C at the slow rate of 1°C/60 hrs. 2. Cold-adapted fishes subjected to a temperature change from 30°C to 20°C at the slow rate of 1°C/60 hrs. 3. Heat-stressed fishes subjected to an abrupt temperature change from 20°C to 30°C at the rate of 1°C/hr. 4. Cold-stressed fishes subjected to an abrupt temperature change from 30°C to 20°C at the rate of 1°C/hr.

At the time of experimentation, the fish were fed normally as described earlier and water in the jars holding the fish was renewed daily for the purpose of cleaning it of
deposits excess food, faecal pellets and so on, and also for replenishing oxygen content therein. The chlorinity and pH of the water used for this investigation was checked from time to time, and was found to be maintained at 0.111 gm/lit and a pH of 7.4 ± 0.1. Since, physiological effects of changes in ambient temperature are known to be altered by other variables, care was taken to control every other factor as far as possible. In this respect, tap water from the same source has been used throughout this investigation for the maintenance of fish and for the experimentation. The fish was starved for 24 hours prior to each estimation so as to eliminate the possibility of differential feedings, handling, especially when sudden, violent and frequent is known to cause laboratory diuresis in fish. Therefore, the experimental fish were handled gently and carefully, to eliminate such effects.