Introduction
Temperature is one of the most important environmental factors with tremendous influence on the Eco-physiology of organism. Results of many studies are available in the literature on various fish-temperature relationships. Fish can perceive water temperature changes, which are smaller than 0.1°C and every fish species has its characteristic acclimatization temperature and temperature tolerance limit, which might change seasonally in a given stock and can be slightly different from one stock to the other of the same species (Laevastu and Murray, 1981). Fishes are to be found inhabitants of environmental temperatures ranging from below 0°C to 50°C. The Antarctic shallow water fish, *Notothemia coriiceps*, can survive at sub-zero temperature of -2.09°C (Hargens, 1971), whereas *Cyprinodon macularius* can tolerate a higher temperature of 52°C (Nikolsky, 1963) and perhaps these are the lowest and highest environmental temperatures respectively at which inhabitation of fishes has been reported so far. The rate of metabolic process in the form of activity of animals is influenced by the ambient temperature. This environmental factor exerts a tremendous influence on the ecophysiology of animal, its activity and distribution (Precht *et al.*, 1973). The distribution of animals has been extending from more uniform temperature conditions of land and waters within the tropics, towards the poles into areas of extreme climatic conditions (Phol, 1976).

An organism is an inseparable part of its environment. Hence, it forms a part and parcel of the environment. Environment being labile, no organism lives under
constant environmental conditions. This environmental lability imposes considerable stress upon the inhabiting organism which in turn have to adjust or adapt to it in order to survive in the extreme environments. If they fail to adapt, organisms succumb to the imposed environmental-stress. And so, the animal has to adapt to the environment in order to survive. Fishes are obligate poikilotherms, some of which can perceive temperature changes of less than 0.5°C (Murray, 1971). Although there is some thermo-regulation by local conservation of muscular heat in at least two groups, the tunas and the lamnid sharks (Fry and Hochachka, 1970; Stevens and Neill, 1978), most of the fishes lack a mechanism to maintain an independent body temperature and the term thermal conformers is invariably applicable to them. Further, temperature and salinity changes in the environment are always challenges to aquatic poikilosmotic animals and their effects are not predictable, since temperature can modify the effects of salinity and salinity can alter the effects of temperature (Kinne, 1964a,b). Thus combined affects of salinity and temperature play an important role in the survival dimensions of the animals (Prakasam and Azariah, 1990).

The adaptation to a new temperature or a change in temperature involves a number of active processes and hence energy expenditure is needed for metabolic re-organisation (Hochachka, 1961a,b; 1967, 1969; Hochachka and Somero, 1968, 1973). The compensation to a change in the temperature in poikilotherms is ultimately exhibited at the individual level, cellular level and sub-cellular level involving the enzyme systems. Further, the concept of poikilotherms regulating their metabolism and activity against ambient temperature variations, though in a limited sense, has
also been established. Extensive work has been done and voluminous data are available in these areas of thermal-adaptation in poikilotherms, especially in fishes (Reviews: Theses Kinne, 1964a,b; Fry, 1964,1970; Prosser, 1965; Pampapathi Rao, 1967; Precht et al., 1973; Hazel and Prosser, 1974; Basha Mohideen, 1979, 1983, 1986; Mushtaq Ahmed, 1988; Abdul Rahim, 1989; Shankar Naik, 1993). In recent times, it has also been known that the similar type of cellular metabolic processes are operated in both poikilotherms and homeotherms. (Anthony and Munro, 1964; Hochachka, 1969; Tarr, 1969). Moreover temperature and body mass effects on the metabolic rate of juvenile, subadult stages of lizard Acanthodactulus at ambient temperatures at 10°C, 15°C, 20°C, 25°C, 30°C and 35°C have been reported by Mohammed and Abdo (1992). These studies indicate that juveniles and subadults were found to have higher rates of oxygen consumption than adults. This is the outcome of several decades of intensive research in the field of temperature-adaptation in cold-blooded animals.

The phenomenon of stress

The concept of biological stress has stimulated numerous formal definitions in the scientific literature, the variety of which bears testimony to the difficulty of establishing a single, comprehensive definition. All the definitions of stress, however share the common precise of a stimulus acting on a biological system and the subsequent reaction of the system. It could be stimulus or response of the system, and a condition that forces a deviation from homeostasis. The concept of stress in relation to biological systems has evoked lot of discussion and disagreement, Pickering (1981)
used the term "Stimulus" for stress and the reaction of the system as the "stress response". The term stress is used inconsistently. However, the following literature reveals the definition of stress and other terms that come under stress and biological investigations on stress.

The term “stress” used by Selye (1950) for the “sum of all the physiological and biochemical responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force”. It is sometimes taken to mean the environmental alteration itself and sometimes the response of fish, population or eco-system (Pickering, 1981). According to (Esch and hazon, 1978), stress is the effect of any environmental alterations or forces that extend to homeostatic or stabilizing processes beyond their normal limits at any level of biological organisation. “Stress” is used to mean the environmental factor itself and a stress factor or “stressor” is an environmental change that is severe enough to require a physiological response on the part of the animal. If the stress response can re-establish a satisfactory relationship between the changed environment and the fish population or ecosystem, adaptation to the stress will occur. However, because of species shift and energy drains which may occur, the original conditions may never be restored (Wedemeyer and McLeavy, 1981). Recently (Rubinsky et al., 1992) have reported the presence of “antifreeze proteins” from the waiterer flounder (Pseudopleuronectes americanus) during cold-adaptation.
Thermal-stress

"Thermal-stress" can be defined as any temperature change that produce a significant disturbance in the normal functions of a fresh water teleost and thus decrease the probability of survival (Elliot, 1981). This definition is similar to that proposed by Brett (1958) and assumes that the response is a stochastic variable that can be measured quantitatively. Several authors used different terminology in studies regarding tolerance and resistance to thermal-stress. Richards et al., (1977) in their review Temperature-preference-studies in environmental impact assessment defined "preferred" or "selected" temperature as the temperature at which fishes are most frequently found when they are allowed to move freely in a temperature gradient. The critical temperature ranges are the ranges over which a significant disturbance in the normal behaviour of fishes may occur i.e., there may be obvious sign of thermal-stress. As most of work on thermal-stress has been to predict the effects of thermal discharge on fish, information on the upper critical range is more numerous than on the lower critical range. The lowest value in the upper critical range is close to the "avoidance", "restlessness" or "disturbing" temperature (Coutant, 1977; Alabaster and Lloyd, 1980), whilst the highest value is the maximum temperature (critical thermal maximum) at which fishes survive for brief periods. The critical thermal maximum is the temperature at which fish loses its ability to escape from the lethal conditions. Low temperature 10°C to 15°C acts as a limiting factor for the noble cray fish Astacus astacus population was reported Pursiainen et al., (1992), studies of this type provide information of aquatic animal stocking in cold-circumstances.
The stress responses of teleostean fishes are considered to be similar in nature to the General Adaptation Syndrome (GAS) proposed for mammalian vertebrates (Selver, 1973). Implicit in this concept is the probability that the health or well-being of fish may be impaired by stress momentarily. One might thus alternately view stress not directly in physiological terms but rather functionally as defined by Brett (1958). The physiological responses to a variety of stress have been outlined for fishes by (Gronow, 1974a,b); Mazeaud et al., (1977); Eddy (1981) and Hughes (1981). Stress has been described as (a) an alarm reaction, in which stress hormones are released, (b) a stage of resistance during which adaptation occurs and (c) a stage of exhaustion, if adaptation is lost because the stress was too severe or long lasting. These three phases cannot be differentiated exactly in all cases. Therefore, many authors refer only to stress effects comprising all symptoms exhibited under physiological load. Further it is widely accepted that the stress response as a whole is characterised by physiological changes, which tend to be similar for stressors as varied as handling, disease treatment, fright, forced swimming, anesthesia, rapid temperature change (or) scale loss, stress response of a fish is analogous in many ways to that occurring in the higher vertebrates (Peters, 1979). The response of a fish to stress is either primary or secondary. Primary responses includes neuro-endocrine and endocrine reactions (Donaldson, 1981; Mazeaud and Mazeaud, 1981; Powers et al., 1982) and the secondary responses to thermal-stress which include disturbances in osmotic and ionic regulations, metabolic processes, growth, reproduction and behaviour. The ultimate response is death (Elliot, 1981). The reactions of a fish to changes in temperature are multiple in number and complex in nature, because
homeostasis with respect to temperature appears to be more critical responses or compensatory acclimations (Prosser, 1965). A terminology on stress in detail is available in the book ‘stress and fish’ (Pickering, 1981). However “stress” is being defined in recent times as physiological load acting upon an animal or man and the factors causing stress are called as “stressors” (Kunnemann and Precht, 1975; Braune and Gronow, 1975a,b; Grigo, 1975; Basha Mohideen and Kunnemann, 1979; Basha Mohideen, 1984, 1989). Similar stress and aggressive responses were also reported in higher vertebrates like mice Moshkin and Frolova (1990). In this study individual differences of calorigenic reaction after cold exposition.

The process of adaptation

The adaptation is a slow process in which the fish adjusts to a slow change in the environment without physiological load on the part of it. Adaptation is the variation that permits biochemical or other activities within a ‘normal’ or ‘middle’ range of environmental factors. Metabolic compensations are particularly well known in acclimation to temperatures. A function may show complete or partial compensation or even over compensation as a result of acclimation. Resistance and capacity adaptations may or may not be related in mechanism, both may be meaningful or one may be paradoxical (Precht, 1958). Further many poikilothermic animals especially fishes respond to changes in environmental temperature with alterations in metabolic rate (capacity adaptation) and with variations in tolerance to environmental extreme temperatures cold or hot (resistance adaptation). The former
enables animals to become independent of the ambient temperature and the later in advantageous to survive in extreme temperatures.

Capacity adaptation is usually measured as the rate of some activity. Developmental rates of cold water animals are faster than those of warm water species at a given temperature. Similar differences are for metabolic rates for some species of fish but not for others and apparently occur rarely in insects. In cold-acclimation, a rate temperature curve may be translated to the left so that at an intermediate temperature the rate for cold-acclimated individuals is higher than for warm-acclimated ones. Or a rate temperature curve may be rotated so that the temperature coefficients are different according to acclimation (Prosser and Nagai, 1968; Prosser, 1973).

It has been discovered that the stress-adaptation results in a gain of response in relation to extremely low and high temperatures i.e., in the process of resistance adaptation (Selye, 1950). The process of resistance-adaptation has been reported in the fish, Rhodeus amarus as a good example (Kunnemann, 1973) where the heat resistance of the fish was determined by a increase in the ambient temperature (1°C/minute), beginning from the adaptation temperature like 10°C and 20°C until cessation of opercular movements. These movements can be regarded as an adequate indicator for heat-resistance (Thiede, 1965). In contrast to resistance-adaptation, hardening appears to be (shock or stronger stress) adaptation which is effected especially by extreme temperatures and hardening also represents a fast adaptation to abrupt changes in temperatures (especially at sub-lethal temperatures, which act as
stressors). In contrast to hardening (Shock or stronger stress), resistance adaptation, however, usually requires several days or even longer period. It represents a long-term adaptation to long-lasting climatic changes (Kunnemann and Precht, 1975). Therefore, when the animals are transferred abruptly to extreme temperatures, either cold or hot, stress results in the form of cold shocks and heat shocks. Therefore it is also necessary to separate the temperature resistance adaptation from greater stress phenomena, in the form of cold shocks or heat shocks.

When poikilotherm is subjected to a relatively long term, persistent thermal stress, adaptive changes are initiated that allow organism to exhibit similar rates of physiological activity in spite of widely different body temperatures. Such compensations occur within the lifespan of an individual of the species and generally require several weeks for completion. This process is customarily referred to as acclimatization if the organism is responding to seasonal fluctuations in the environmental parameters within its natural ecosystems or acclimation if the organism is responding to a single, well defined environmental parameters under a laboratory situation. Physiological compensations associated with a period of acclimation or acclimatization are common among poikilotherms and have been reported for all levels of biological organisations.

Thus many poikilothermic animals respond to changes in the environmental temperature by altering the metabolic rate within the normal range of temperature. Recently, it has been discovered that 'temperature-stress' is entirely different from 'temperature-adaptation' (Laudien and Phol, 1975; Kunnemann and Basha Mohideen,
1976, Basha Mohideen and Kunnemann, 1979; Basha Mohideen, 1984; Mushtaq Ahmed, 1988 and Abdul Rahim, 1989). An abrupt temperature change results in the process of stress condition. "Stress" is a physiological load acting upon an animal or man, and the factors which induce stress are called the "stressors". Conversely, when the animals are exposed very slowly to a new temperature, they compensate, resulting in the process of adaptation without physiological load on the part of the animal (Basha Mohideen, 1979, 1984; Basha Mohideen and Kunnemann, 1979). Physiological load means an undue burden on the animal with rapid or steady release of energy (Brett, 1958). Fry (1957, 1964) classified environmental effects into five groups of factors viz., lethal, controlling, limiting, masking and directive. This approach was followed by Brett (1958) who divided stresses on fish into four categories; lethal (self-explanatory), limiting (restriction in the supply of essential metabolites or interference with the chain of energy release), inhibiting (reduction in the ability of the fish to carry out its normal functions and hence a reduction in its probability of survival) and loading (an induced burden on the fish with a rapid or steady release of energy). Limiting, inhibiting and loading stresses may also be lethal when they continue over longer periods.

Thanks to the pioneering works of Precht and his collaborators of the new approach on adaptation process and the year 1975 heralded the beginning of a new era of approach of differentiation of these thermal-stress phenomena from thermal-adaptation processes where, it is possible and necessary to distinguish the two different processes of "stress" and "adaptation", when poikilothermic animals are
subjected to thermal changes and when the process of acclimation is complete, one has to differentiate between possible thermal-stress and thermal-adaptation (Kunnemann and Precht, 1975; Kunnemann and Basha Mohideen, 1976; Basha Mohideen and Kunnemann, 1979; Basha Mohideen, 1979, 1982, 1983, 1984, 1985, 1986, 1987a,b,c and 1989). Based upon the above factors, in the present investigation, the author has made a humble attempt to differentiate the adaptation process from the stress phenomena and their effects on the fish in recovering from the physiological load and is caused by the stress conditions, towards heat as well as towards cold and ultimately to decipher the mechanisms especially the energetics involved during temperature-stress and temperature-adaptation processes, using the following parameters - Physiological and biochemicals.

**Time course of thermal-adaptation**

Many eurythermal poikilotherms are able to survive and function at widely different habitat temperatures. This is largely related to the fact that when a poikilotherm is transferred from one thermal environment to another, many aspects of its physiology and biochemistry are altered in a manner that often compensates the temperature change. Such compensations to temperature may occur over at least three distinct time course periods (1) Temperature compensation appears to be instantaneous, (2) Compensation is evident in an individual of species only after a period of acclimation and (3) Thermal compensations may become evident over evolutionary time spans (Somero, 1969; Hochachka and Somero, 1973). Physiological responses to the imposed environmental stress may be thermal osmotic
or any other factor may be divided into two categories (1) Immediate or short term responses when the animal is transferred immediately to the stress medium and (2) Stabilized or long term responses when exposed to prolonged period in the stress medium. This prolonged period may be hours, days or weeks or even evolutionary span in different cases of animals (Grainger, 1958; Kinne, 1958, 1960, 1964a,b, 1966, 1967, 1971; Parvatheswara Rao, 1968a, Somero, 1969; Somero and Hochachka, 1969; Hochachka and Somero, 1973; Hazel and Prosser, 1974; Basha Mohideen and Parvatheswara Rao, 1976; Basha Mohideen and Kunnemann, 1979; Basha Mohideen, 1982, 1984, 1987a,b,c).

Thus when poikilotherm is adapted to a new thermal regime over a period of time which varies from animal to animal, its metabolic rate after a period gradually diminishing fluctuation, stabilizes at a new rate, which is maintained duly its subsequent sojourn in the new thermal regime, and the attainment of such stabilized rate is said to signal the completion of adaptation (Kinne, 1964a,b). However a relationship between the stabilized rate in the new thermal regime and the original normal rate in the natural habitat. Temperature differs between different poikilotherms depending upon the differences amongst them in the degree and the direction of metabolic thermal-stress. According to Precht, 1958, has classical metabolic adaptation to thermal-stress into 5 different patterns- (1) Over adaptation: where the new stabilized rate of metabolism in the adaptation medium gives beyond the original normal rate of the natural temperature, (2) Complete adaptation: where the new stabilized rate of metabolism in the new thermal regime perfectly coincides
with the original normal rate in the natural habit, (3) Partial adaptation: where the new stabilized rate of metabolism is half way between the initial shoot-up or shoot-down level as the case may be, (4) No adaptation: where the deviated metabolic rate is continued to be maintained even after prolonged exposed to new thermal regime and (5) Reverse adaptation: where the metabolic rate, unlike in normal adaptation continuous to increase or decrease beyond the initial shoot-up or the initial shoot-down level as the case may be during the subsequent sojourn of the poikilotherms in the adaptation medium.

In most of the poikilotherms adaptation of metabolism to thermal stress follows patterns 2 and 3 and this is repeated in several cases of fishes (Precht, 1951, 1958; Evans et al., 1962; Fry, 1964; Morris, 1965; Peterosn and Anderson, 1969; Parvatheswara Rao, 1971a, 1972b; Campbell and Davies, 1975; Kunnemann and Basha Mohideen, 1976; Paravathi, 1982; Mushtaq Ahmed, 1988; Abdul Rahim, 1989; Dhanunjaya, 1990). However the patterns of adaptation may be different in the same for adaptation to cold and warm temperatures (Parvatheswara Rao, 1972a,b; Mushtaq Ahmed, 1988; Abdul Rahim, 1989).

In several fishes, it was reported that adaptation to higher temperature proceeds faster than adaptation to lower temperature. Therefore in fishes increases in heat resistant through warm adaptation is faster than the loss of it or gain in cold resistance through cold-adaptation ( Spoor, 1955; Brett, 1956). Studies of this nature on time course of adaptation to thermal-stress in tropical fishes has so far received very little attention. Hence, a study of time course of metabolic adaptation to cold
and warm temperature is tackled in this investigation and such studies form a good thermal background for further studies of temperature differentiation of thermal-stress from thermal-adaptation.

In most of the fishes, it was reported that adaptation to higher temperatures proceeds faster than at the lower temperatures. This reflects in report that, in fishes, increase in heat-resistance through warm adaptation is faster than the loss of it or gain in cold resistance through cold-adaptation (Spoor, 1955; Brett, 1956) but also the operation of different mechanisms during adaptation to cold and warm temperatures (Fisher, 1958; Konishi and Hickmann, 1964; Vernberg and Vernberg, 1966a; Paravatheswara Rao, 1967, 1968a,b). It was first suggested in the studies of Summer and Sargent (1940) in which warm adapted fishes, *Cyprinodon* and *Crechthys* were found to succumb to potassium cyanide treatment for more easily than cold-adapted ones, indicating the predominance of cyanide sensitive metabolic pathway in the warm adapted fishes.

According to Arrhenius (1889) in his theory predicts that an increase in temperature will increase the rate of chemical or biochemical reactions. A 10°C rise in temperature will result in an approximate doubling of the rate of most biological processes ($Q_{10} = 2$) although overall range of $Q_{10}$ values for biological processes appears to be between 1 to 3. This relationship is of fundamental importance to the poikilotherms because, changes in the environmental temperature will dictate corresponding changes in the metabolic rate, although in course of time, adaptive processes may take place. General aspects of this subject are discussed by Prosser
Further, the sequence of events in time course of adaptation may reflect, corresponding changes in the cellular enzymes (Parvatheswara Rao, 1968a; Sidell et al., 1973). Studies on time course of adaptation to temperature-stress in tropical fishes has so far received little attention, despite its importance in the understanding of molecular changes, triggered by the imposed temperature-stress (Hazel, 1972). When a poikilothermic animal is transferred to a new ambient temperature, its metabolic rate shoots-up initially, but after a prolonged time stabilises at a new rate and the attainment of such stabilized rate is an indicator for the completion of adaptation (Kinne, 1964a, b; Parvatheswara Rao, 1968a, 1970; Basha Mohideen and Parvatheswara Rao, 1976; Precht, 1958), considered and classified these studies involving the attainment of the new stabilised metabolic rate in the new thermal environment into five distinct thermal acclimation patterns in poikilotherms. Adaptative physiology of *Catla catla* during thermal biology has been worked out by Sujatha (1997).

**Temperature-adaptation - Oxygen consumption of the whose fish**

Measurement of oxygen consumption have been employed more than any other experimental parameter to monitor changes is metabolism associated with temperature-adaptation. The uptake of oxygen rate is considered as a good index for overall physiological activity and an indicator of environmental-stress of the animal, and such a physiological index is easy to obtain both in the field and in the laboratory with a variety of environmental stresses, including the temperature-stress of the present investigation. Further the variation in oxygen consumption can be accounted
for the excellent modulation in the metabolic status of the animal (Natarajan, 1980; Basha Mohideen, 1984). When the fish is abruptly transferred from warm to a cold environment, oxygen consumption drops immediately due to direct kinetic effect of temperature on the reactions involved. After stabilized phase which may be several hours or days in duration. The oxygen consumption rises and eventually stabilises at a value characteristic of the cold-adapted state. These fluctuations result due to the operation of stress phenomena (Sidell et al., 1973). Due to the effect of temperature on the oxygen solubility, an aquatic poikilotherm exposed to elevated temperature is confronted with a much increased oxygen demand in an environment that is relatively hypoxic (Hazel and Prosser, 1974). Chi and Adelman (1990) reported temperature acclimation in respiratory and Cytochrome-c oxidase activity in common carp *Cyprinus carpio*. These findings suggest the low cytochrome-c oxidase activity at a cold acclimation of temperature 12°C was probably due to thermal compensations and the high activity at 30°C might have been resulted due to thermal-stress.

The rate of metabolism of the animal is considered to be a good indicator of thermal-stress in animals, and measurement of oxygen consumption has been employed more extensively than any other metabolic parameter. The studies on metabolic adaptation to temperature, indicate that when poikilotherm is adapted to a new thermal range over a period of time, which varies from animal to animal, its metabolic rate, after a period gradually diminishing fluctuations, stabilises at a new rate, which is maintained during the subsequent period in a new thermal range (Kinne, 1964 a,b). The relationship between the stabilised rate in the new thermal
range and the original normal rate in the natural habitat temperature differs between different poikilotherms depending upon the differences among them in the degree and direction of metabolic adaptation to ambient temperature. Precht (1958) has classified the metabolic adaptation to temperature into five different patterns as given in detail on the earlier section.

In most of the poikilotherms, adaptation of metabolism to temperature follows pattern 2 and 3, and this is reported in several cases of fishes such as eel, *Anguilla vulgaris* (Precht, 1951), the rainbow trout *Salmo gairdneri* (Evans et al., 1962), yellow bull head *Ictalurus natalis* (Morris, 1965), the Atlantic salmon *Salmo salar* (Peterson and Anderson, 1969), the toad fish, *Opesanus tan* (Haschemayer, 1969), and *Blennius pholis* (Campbell and Davies, 1975). However, the pattern of adaptation may vary in the same fish at different periods depending upon the differences in the thermal history and further, in the same fish, adaptation to cold and warm temperatures may follow different patterns (Parvatheswara Rao, 1968a, 1971a, 1972a,b). It is interesting to note that adaptation to temperature involves a number of active processes, making additional energy demands. The primary purpose of metabolic regulation during adaptation to temperature might be to meet such energy demands. However, studies in this direction are very few in fishes. Most studies on fishes in this area are confined to comparing oxygen consumption of the cold-adapted and warm-adapted fishes at a few common intermediate temperatures (Scholander *et al.*, 1953; Stronganov, 1956; Winberg, 1956; Wohlschlag, 1963; Kanungo and Prosser, 1959a,b, Roberts, 1960, 1967; Haschemeyer, 1969; Parvatheswara Rao,
Studies of this nature are not quite sufficient in the understanding of energetic aspects of thermal-adaptation as animals are often exposed to sudden changes in environmental factors, which results in the form of cold shocks or heat shocks. Therefore, it is necessary to separate the temperature resistance adaptation from greater stress phenomena in the form of cold shocks and heat shocks.

It has commonly been supposed that the increased oxygen uptake is due to the extra work of breathing following the hyper-ventilatory responses. The valuable estimates (Hughes, 1973; Jones and Schwarzfeld, 1974) suggest that 10% of the standard metabolism is required for the ventilatory movements under these conditions. Many fishes, especially the fresh water fishes, on account of their organisation and higher metabolism, have greater need for oxygen than the others. If the Minnows come to the surface, it is not only to catch the files, but to have more oxygen available in the surface water. If they keep down below, they perish. The Dipnoi, though provided with gills, must also have free access to the air. Other fishes come up regularly to the surface and taking-in gulps of air and from this habit several important organs have developed (Harry, 1926).

In recent times, it was found necessary and possible to distinguish the environmental-stress from environmental-adaptation, may be thermal as in the case of "temperature-stress and temperature-adaptation". Hence in this investigation, an attempt is made in this direction and to that end heat-stress and heat-adaptation as well as cold-stress and cold-adaptation rate of oxygen consumption is measured in the
whole fish at a gradual change in temperature (adaptation) and compared separately to the sudden and abrupt change in the temperature (stress) in both from cold to heat (heat-stress and heat-adaptation) as well as from heat to cold (cold-stress and cold-adaptation) directions. In this regard, the preliminary work on differentiation of "stress" from "adaptation" process in relation to the environmental temperature has been carried out in the fish, *Idus idus* (Laudien and Pohl, 1975; Kunnemann and Basha Mohideen, 1976, Basha Mohideen and Kunnemann, 1979), in *Cyprinus carpio* (Basha Mohideen, 1984), in *Tilapia mossambica* (Mushtaq Ahmed, 1988) and in *Cyprinus carpio* (Abdul Rahim, 1989).

**Temperature-adaptation - Opercular activity**

The respiratory surface in all animals has in common the presentation to the external medium of a large surface area, often well supplied with blood vessels across which diffusion of gases can occur. The typical respiratory organ gills of aquatic animals including fishes have a large surface area in relation to their weight and pumping devices are often employed to renew the surrounding medium. The gills of fishes are supplied by a series of gill arches housed within a chamber formed by bony plates, the operculum, which is opened and closed rhythmically during respiratory movements. In fishes deoxygenated blood from the heart-flows in the lamellae in a direction counter to that of the water flow across the exchange surfaces, this mechanism is called counter-current principle through which fish derives maximum amount of oxygen from water always fresh and rich in oxygen. The organs involved in the ventilation of a teleostean fish consists of the mouth cavity, the pharynx with its
gills, and the gill covering or operculum. When a fish respires, the initial movement consists of an expansion of the hoop-like gill arches, as a result of which the mouth and pharynx enlarge. The mouth opens, and a stream of water is drawn into the oral cavity, while the external gill opening is tightly closed by the thin membrane bordering the operculum. These steps constitute the inspiratory phase.

The expiratory phase which follows immediately, consists of the closure of mouth and at the same time the contraction of the pharynx, thus directing the water towards the gills and hence through the operculum. This constitutes the ventilatory pump. Thus, the fish's respiratory efficiency depends upon the rate of opercular movements. It has been pointed out that the pulsatory movement of the opening of the mouth and operculum alternates with their closing, and it is easy to watch a fish in an aquarium (Chandy, 1970). Under environmental-stress, the opercular movement can be taken as an index model to verify the respiratory activity of the animal (Thiede, 1965; Narasimha Murthy, 1983; Basha Mohideen, 1984). Further, according to Thiede (1965), the opercular movements can be regarded as an adequate indicator for heat-resistance in fishes. Adeney and Hughes (1977) have given the mechanisms of respiratory system with special reference to gills and branchial circulation in fishes and of water exchange across fish gills (Christopher, 1979). Studies involving the rate of opercular movement are highly conspicuous in fishes and available work on opercular movements in fishes is confined to environmental temperature, where the process of resistance-adaptation has been reported in the fish, Rhadolus amarus as a good example (Kunnemann, 1973), where the heat-resistance of the fish was
determined by an increase in ambient temperature (\(1^\circ C/\text{minute}\)), beginning from the adaptation temperature like 10°C and 20°C until cessation of opercular movements. Adjustments in extraction efficiency and ventilatory rate may also be involved in the acclimatory response and these factors, in continuation, may fully compensate for the increased oxygen tension with acclimation temperature (Jankowsky, 1968). It is clear that fish shows many adaptive responses to lowering of oxygen in the inspired water. Most of these responses are concerned with the ventilatory system and are reflex in nature, initiated by exteroceptors probably on the surface of the gills or the associated regions such as the pseudobranch. There is also evidence that there may be introceptors which respond to changes in blood oxygen levels after it has left the gills and circulates to the brain (Hughes and Ballintijn, 1965). Ventilatory responses mainly serve to increase the total amount of oxygen brought into contact with the gas exchange surfaces (Hughes, 1981) and are also good indicators of stress situation in fishes. Thus oxygen consumption and opercular activity, when considered together act as good indicators of pollution-stress including thermal-stress in fishes (Basha Mohideen and Saila Bala, 1992).

**Temperature-adaptation - Studies on haematological parameters**

In most of the animals including fishes, blood being the medium of internal transport that comes in direct contact with various organs and tissues of the body, the physiological state of an animal at a particular time was reflected in its blood. Studies on blood parameters have become an important diagnostic tools in medicine over many years and they equally serve as diagnostic indices to investigate disease or
stress in fish (Bensol et al., 1979). Studies on fish haematology have drawn a new attention with reference to its basic physiology and also haematological responses to environment (Siddique et al., 1970; Rao and Behra, 1973; Dub and Dutta, 1974; Raizada and Singh, 1982; Basha Mohideen, 1984). The effect of thermal acclimation on blood composition of gold fish (Anthony, 1961); rainbow trout (Houston and Cry, 1974), Carp (Houston and Dewilde, 1968, 1969); frog (Krishnamurthi and Shakuntala, 1974) and side winder (McMohan and Hammer, 1975) and Cattle (Olbrich et al., 1972) has been reported. Several workers have reported the haematological changes in fishes with reference to pesticides (Baynee et al., 1980), salinity, oxygen or carbon dioxide tension on altitude and migration (Brown, 1957; Pradhan, 1961) reported blood corpuscles, haemoglobin concentration and packed cell volume in Tilapia and Ophiocephalus in relation to length and weight. There are several reviews on haemoglobin and the respiratory function of the blood of plasma (Redfield, 1938; Krogh, 1941; Maxwell, 1960; Roughton, 1964; Riggrs, 1965; Antonini, 1965, 1967). In migratory fishes, when they entr fresh water from sea, the blood becomes diluted and the number of red cells per unit volume decreases, hence the oxygen carrying capacity declines (Benditt et al., 1941). The temperature probably affects the annual cycle of oxygen concentration of red blood cells in circulation. Houston and Murad (1992) have reported the erythodynamics in gold fish Carassius auratus where gold fish acclimated to cold-temperature of 15°C and higher temperature of 35°C exhibited minimal concomitant increase in blood viscosity and cardiac work through partial elimination and replacement of preexisting erythrocytes to a greater extent at higher temperature than at lower temperature.
Smirnova (1965) found that the concentration of haemoglobin in the blood of fresh water species from the Rybinsk reservoir is the highest in January, when the concentration of oxygen is lowest and decreases under the more favourable summer conditions. More oxygen dissolves in cold water than in warm, so that at lower temperatures fish have less need for haemoglobin. Platner (1950) noticed that the blood of *Carassius auratus*, exposed to freezing temperatures in aerated water becomes diluted so that the haematocrit blood cell volume goes down. Scholander and Vandon (1957) showed that the iron content of the blood of two cold water Arctic species, out of four, examined was low. Ruud (1954) showed that the Antarctic fish, ice-fish or white crocodile fish (*Chaenocephalus aceratus*) is white and the colour of the gills and blood is also white and no haemoglobin in the blood. For the gaseous exchange, the gill surfaces are large, and Ruud (1958) suggested that skin respiration takes place in these fishes.

The oxygen requirements of a fish will vary according to its activity. The blood of active fish contains more haemoglobin than that of a sluggish fish. Hall and Gray, 1929; Roots, 1931; Hunn and Robinson, 1966) in order to increase its oxygen capacity. Engel and Devis (1964) measured the blood cell volume and haemoglobin concentrations of active and inactive fish, where the inactive fish showed lowest value, while the blood of active fish species possesses a greater number of red blood cells per unit volume, the oxygen capacity of the haemoglobin itself appears not to change but earlier workers (Black, 1940) suggested that, it increased with the activity of the fish. Starvation causes a reduction in the population of different cells in blood
plasma. Small Wood (1916) observed that RBC count of *Amia calva* dropped from 1,640,000 per cubic mm to 400,000, after starvation for 20 months. Murrachi (1959) showed in *Cyprinus carpio* an increased red cell sedimentation rate after starvation for 7 weeks at 6°C to 22°C and a decrease in red cell volume from 50% to 33% with a corresponding decrease in haemoglobin content from 11 to 7 g/100 ml. Higginbotham and Mayer (1948) observed a reduced cell volume, haemoglobin content and blood specific gravity in *Ictalurus punctatus*, where the fish were in poor condition (Emaciation). A decline in the cell number is to mean that the fish is unable to manufacture new blood cells under starved conditions. During the hypoxic conditions swelling of the erythrocytes and an increased haematocrit value (cell count) were observed in the rainbow trout (Holeton and Randall, 1967). The ratio of blood hemoglobin concentration to the haematocrit value is not dependent on the blood volume or on the number of red cells per unit volume (Soivia and Nikinmaa, 1981). However, new cells are liberated into the circulation during anaemia induced by bleeding (Lane, 1979; McLead *et al.*, 1978). They only appear in the circulation 3-4 days after bleeding hypoxia (McLead *et al.*, 1978). Further, the hypoxia induced changes in the weight of the spleen, probably the most likely site for the liberation of erythrocytes (McLeod *et al.*, 1978), representing only 2% increase in the blood volume (Soivio *et al.*, 1980).

The changes in Mean Corpuscle Haemoglobin Content (MCHC) due to the different sampling method indicate that erythrocytes obtained by cardiac puncture from stunned fish had swelled by about 10%. In addition to MCHC values of 6-day
hypoxia at 18°C, indicates a marked swelling of the erythrocytes. During severe hypoxia, marked increase in swelling of the erythrocytes was preserved by Holeton and Randall (1967). In hypoxia, exposure at 11°C, the swelling of erythrocyte was transient (Soivia et al., 1980). At 11°C water temperature, the changes in oxygen affinity is based totally on the decrease in ATP/Hb ratio and not on the changes in the red cell volume. However, at high temperatures (18°C) swelling of the erythrocytes may also decrease the erythrocytic-ATP concentration, which influence the blood oxygen affinity by increasing the intra-erythrocytic pH and this response takes place immediately at the on set of stress, acute hypoxia (Soivio et al., 1980).

The haematological changes that are proving to be useful as indices include, quantifying the decrease in blood clotting time and changes in the differential leucocyte count (McLeay, 1975b; Casillas and Smith, 1977). A decrease in the number of leucocytes (Leucopenia) has recently been found to be a significant part of the physiological response to acute stressors such as crowding (McLeay and Gordon, 1977). Changes in the erythrocyte counts or haemoglobin levels are most useful as indicators of haemo-dilution or haemo-concentration, although anaemias or stress polythermia may occasionally occur (Soivia and Oikari, 1976; Soivio and Nikinmaa, 1981).

When the fresh water teleost fish is exposed to hypoxia, it overcomes the oxygen demand, by increasing the oxygen carrying capacity of blood after warm acclimation. Studies of Houston and Dewilde (1969) on common carp acclimated to 2,4,7 and 33°C revealed a direct correlation among red cell number, packed cell
volume and haemoglobin content of the blood and acclimation temperature. Further, RBC as a good indicator of thermal-stress in Indian major carp, *Catla catla* was reported (Basha Mohideen and Sujatha, 2001).

Therefore, the variations in the basic and highly useful haematological parameters like RBC number are valuable indicators of stress and are also used to evaluate the changes in metabolism due to environmental alterations. But studies on RBC and WBC count regarding temperature-stress and temperature-adaptation are conspicuous by their absence and such studies on haematological parameters like RBC number, WBC number and haemoglobin content obviously form quick and good indicators of stress situation.

**Temperature-adaptation - Energy sources**

Carbohydrates are the most readily utilised and the first to be affected by environmental change. Carbohydrates are stored in the liver as glycogen, a polysaccharide built of glucose units. When required, the glycogen is broken down and transported as glucose, and thus glucose is found in the blood. Generally blood glucose level has been reported as a reliable and sensitive indicator of environmental stress in fishes (Silbergeld, 1974). Extensive work has been done on blood glucose, liver and muscle glycogen in fishes with reference to salinity and temperature (Dean and Goodnight, 1964; Pickford *et al.*, 1969; Umminger, 1971a,b,c; Basha Mohideen and Parvatheswara Rao, 1971, 1972). Umminger (1975) showed that blood glucose is considered to be the good indicator of environmental variations. Further, there is linear relationship between the level of blood glucose and the activity of the animal.
(Vernberg and Gray, 1953; Fakuda, 1958) and the level of blood glucose is attributed to an indirect level of basal metabolic rate in fishes (Umminger, 1977). Thus blood glucose level indicates most striking alterations in response to the changes in environmental factors (Umminger, 1975; Hattingh, 1977). General account on the energy relations of animals was reported by Kleiber (1961). Stimulatory effects of cold exposure on the glucose uptake in rat peripheral tissues induces regulatory alterations in glucose metabolism (Vallerand et al., 1991).

Early work in the carbohydrate metabolism of fishes was confined to exercise effect, post-exercise recover and effects of hypoxia (Black, 1940; Black et al., 1960, 1961; Blazka, 1958; Hochachka, 1961a,b; Hochachka and Sinclair, 1962; Ekberg, 1962; Dean and Goodnight, 1964; Heath and Pritchard, 1965; Bellamy, 1968) found that the liver glycogen of Roosevelliella vattereri is rapidly mobilised during starvation, falling from 10.3% to 0.5% only after 8 days. In contrast, the glucose in blood of several species is maintained at a steady level during long periods of starvation. Studies on Salvelinus fontinalis, the blood glucose decreases during the first 3 days, but remains steady thereafter (Phillips et al., 1953) and similarly, the blood glucose of Godus morhua declines from 108 mg% to 72 mg% in the first 37 days but remains constant after 51 days of fasting at 6.5°C (Kamra, 1966). Many workers found that blood glucose level has been correlated to their level of activity and hence to their level of metabolism. It has been reported that blood glucose level is low in sluggish fishes and it is relatively high in active fishes. (Gray and Hall,
Maintenance of glycogen reserves is one of the most important features of the normal metabolism (Mong and Polond, 1981). Alterations in liver glycogen under situation of stress has been reported and a significant depletion in tissue glycogen is said to reflect a state of strenuous activity on the part of the fish (Tewari et al., 1987; Vijayaram et al., 1989). Studies of similar nature have shown significant decrease in the level of muscle glycogen which is said to reflect a state of strenuous activity on the part of the fish (Black, 1955; Miller et al., 1959; Black et al., 1960, 1961, 1962; Basha Mohideen and Parvatheswara Rao, 1971, 1972, 1973). In the Pacific Killifish, Fundulus heteroclitus, glycogen phosphorylase activity is increased at lower temperature and this was accompanied by decrease in hepatic glycogen level, suggesting increased hepatic glycogenolysis at low temperatures (Umminger, 1970b, 1975). It has long been known that different kinds of stress (anoxia, struggling etc.) induced an elevation of blood glucose in elasmobranchs (Scott, 1921) in teleosts (McCormic and McLeod, 1925; Love, 1970) and such a capture and handling almost result in hyperglycemia (Chavin and Young, 1970; Weedemeyer, 1972; Miles et al., 1974; Specker and Schreck, 1980). The lactate-pyruvate is a good indicator for stress situation in the dorsal muscle of the fish golden orf Idus idus (Gronow, 1964a,b; Braune and Gronow, 1975).

Other studies on stress shows glycogen break down within seconds in the muscle and within hours in the liver and this is usually followed by a prompt recovery
in the liver with some variations (Love, 1970; Narasimhan and Sundraraj, 1971; Miler et al., 1974; Plisetskaya, 1975; Wardle, 1978). Capture and handling, and anesthesia all have profound effects on the carbohydrate metabolism and blood electrolytic balance. These changes are thought to be under the endocrine control (Mazeaud and Mazeaud, 1981; Donaldson, 1981). Thus, there is a great deal of work and voluminous data in carbohydrate metabolism are available during environmental-adaptation in general. But highly inadequate information on carbohydrate metabolism is available on temperature-stress and temperature-adaptation in fishes. Hence an attempt is made to study carbohydrate metabolism involving blood glucose, liver glycogen and muscle glycogen contents during abrupt and slow changes in the environmental temperature (both towards heat i.e., from 22°C to 32°C and towards cold i.e., 32°C to 22°C) to differentiate the temperature-stress phenomena from temperature-adaptation processes.

**Temperature-adaptation: Adenylate nucleotides and Energy charge**

The most important adenylate nucleotides that are available in the animal body are ATP, ADP, AMP and energy charge. The energy relationships of these different nucleotides are quite different from one other. The hydrolyses of the diphosphate and triphosphate bonds are associated with the release of large amount of free energy—approximately 8000 cal/mole, while the phosphate bond of adenylic acid (AMP) is associated with only 1000 or 2000 cal/mole. Therefore, the former are referred to as high energy or energy-rich compounds, while the latter is called as low energy or energy-poor nucleotide. Most of the specific task of the animal such as concentration
or secretion depends directly on the ATP-ADP phosphate transfer system and for some biosynthetic processes, the phosphate bond energy comes from the hydrolysis of ADP to form AMP (Hoar, 1991). In fact, the energy status of the cell depends on the relative amounts of those three derivatives of adenosine. As a primary energy source, these phosphate compounds serve as allosteric modulators in many important metabolic reactions, for example, the allosteric enzyme phosphofructokinase, concerned with the control of glycolysis in the interconversion of Fructose-6-phosphate and Fructose-1-6-diphosphate is strongly inhibited by ATP but stimulated by AMP and ADP, while fructose diphosphate phosphatase is strongly stimulated by ATP and inhibited by AMP (Hoar, 1991). Atkinson (1970) refers to ATP – ADP-AMP status of a cell as its energy charge. If this system is filled or fully charged with high-energy phosphate, then all the adenosine nucleotide is in the form of ATP and the charge is 1.0. On the other hand, if all the nucleotides is in the form of AMP, the system is ‘empty’ of low energy phosphate and the charge is 0. At an average charge of 0.5 all the adenosine compounds will be in the form of ADP or an equimolar mix of the ATP and AMP. The energy charge in the cells of normal fasting mammals is about 0.85. A further detailed account of this concept is seen in Atkinson (1970, 1977), Lehninger (1975), Basha Mohideen and Kunnemann (1978) works. Therefore the adenylate system is considered as standard, wherein the energy charge is defined as follows: (Atkinson and Walton, 1967). An increase ATP and energy charge of Atkinson was observed in liver mitochondrial respiration during hyperthermia Imoto and Yuko (1990).
Adenelyte energy charge = \( \frac{\text{ATP} + \frac{1}{2} \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}} \)

Thus, the energy charge is one of the regulatory inputs to most, and probably all metabolic sequences even a slight decrease in secondary, and more indirect effects to change in the rates of most of the metabolic sequences and in the concentration of many metabolites is brought about (Alkinson, 1968). The values on the concentration of the adenylate-nucleotides in the case of various species have been tabulated by Bonsel and Pradet (1968), Chapman et al., (1971), Beis and Newsholme (1975). In most of these cases, the energy charge lies between 0.75 and 0.98. An extremely lower value for the energy charge of 0.1 was reported, (Ball and Atkinson, 1975). Further Bertin et al., (1990) also reported the nature of energy metabolism during cold-accimilation and this may provide a good model for the study of the cold-induced mechanisms.

The variations in the concentration of the adenylate nucleotides like ATP, ADP, AMP and energy charge have been reported by a few investigators in relation to different environmental conditions in the case of fishes. Ivanovici (1980) has studied the adenylate energy charge in the estuarine mollusc, Pyrazus abeninus in relation to ambient salinity and temperature indicated that the speed and precision of response by adenylate energy charge may be useful in monitoring the effects of environmental perturbations. Dickson and Sprangue (1981) have shown that the ATP turn over and adenylate energy charge in excised gills was known to differ significantly between surface and cave Cray fish. Comselle et al., (1980) reported that the concentrations of
adenylate nucleotides and available energy charge in the hepatopancreas of *Mylilus edulis* is found to vary significantly in different experimental conditions like air-exposure, cold-assimilation, glucose-feeding, alanine feeding and succinate feeding Kunnemann and Basha Mohideen (1976), Kunnemann *et al.*, (1978); Precht *et al.*, (1973); Kunnemann and Precht (1975); Basha Mohideen, (1979, 1983, 1985). Further extensive work has been carried out on the adenylate nucleotides like ATP, ADP, AMP and energy charge in the brain, dorsal muscle of the fish, *Idus idus* in relation to environment temperature by Basha Mohideen and Kunnemann (1979). These studies clearly indicate that there is a steady increase of energy charge in the muscle and brain of the fish, *Idus idus* in the range of adaptation temperature from 5°C to 20°C. Further, the fishes subjected to a very slow temperature change at the rate of 1°C / two days from 10°C to 20°C, there is filling-up processes with ATP, with reaching of the controls in these nucleotide concentrations; whereas the fishes which are abruptly exposed to environmental temperature from 10°C to 20°C at the rate of 1°C/hr, the filling up processes with ATP could not be observed and there is no reaching of the control in these adenylate nucleotide concentrations. Thus these studies on nucleotide metabolites clearly establish that temperature-adaptation is different from temperature-stress in that, stress is a physiological load acting upon an animal or man and factors causing stress are called as stressors, whereas adaptation is a slow process of compensation without physiological load. Such an environmental differentiation could well be established elaborately involving these energy nucleotides.
There are few studies involving the adenylate nucleotides and energy charge with reference to environmental-pollution, further these are only confined to chlorinated hydrocarbons that too mostly confined to insects and mammals. The first research on adenosine triphosphate (ATPase) showed no adverse effect resulting from DDT (Gonada et al., 1959; Judah, 1949), although in the latter study the inhibition of oxidative phosphorylation was demonstrated by using 100 μ moles DDT. However, in 1969, the first report on the inhibition of ATPase by organochloride pesticides was made by Koch (1969a,b). Recently (Tang et al., 1990) studied the inhibitory effects of permethrin on calcium and magnesium, ATPase activity of rat brain in synaptosomes. (Hohreiter, et al., 1991) studied the effects of carbofuran and fenevelerate on adenylate parameters in blue gill sun fish Lepomis macrochirus. Very recently, Basha Mohideen et al., (2001) have reported compensatory changes in energy budget in the tissues of Cyprinus carpio during thermal-stress.