Chapter-2

REVIEW OF LITERATURE

Effect of oxygen deficiency on fish had drawn the attention of scientists as early as 1920s and extensive literature is available on fish during that period (Gardner, 1926).

Story of studies of adaptations of fish to low oxygen was extended by investigation undertaken in swamps (Carter and Beadle, 1931). It observed in 1940s that fishes suffered the effect of hypoxia in periods of extreme winter in the lakes of North temperate zones (Greenbank, 1945; Cooper and Washburn, 1949).

That low oxygen is a major stress in the environment was inferred by the extensive researches of Jones (1952). In the process of determining the water quality parameters of freshwaters and marine species of Canada, the importance of critical need of oxygen were under linked (Davis, 1975). Earlier, around 1950s, episodes of mass mortalities of marine organisms due to oxygen depletion had occurred (Brongersma-Sanders, 1957). Incidences of migration of organisms due to hypoxic condition to the surface of the water, like one that happened in Mobile Bay (Alabamas) (Loesch, 1960) drew attention of Scientists towards effect of environmental hypoxia and during 1970s and 1980s there was a fleet of investigations to analyze the various manifestations of fishes in response to varied level of oxygen in the environment. The laboratory investigations revealed that the manifestations take place at all the three levels of fish- namely behavioural, physiological and biochemical. Behavioural studies are well documented.
Kutty (1968) and Bushnell et al., (1984) investigated the effect of chronic hypoxia on fish swimming performance and metabolism. The effect of hypoxia on swimming activity of fishes was supported by Dahlberg et al., (1968), Kutty, (1968), Bushnell et al., (1984), Dutil and coworkers (2007) investigated swimming performance of fishes during different periods of hypoxia.

A comprehensive study has been made on a number of freshwater, estuarine and marine fishes by Davis (1975) to record the minimum oxygen requirements for survival and growth of fishes.

Greaney et al., (1980); Taylor and Miller, (2001); Pichavant et al., (2003) studied the effects of chronic (weeks of) hypoxia on oxygen carrying capacity.

In 1983 Weber & Kramer described that feeding and growth (Cech et al., 1984; Bejda et al., 1992; Hales & Able, 1995; Secor & Gunderson, 1998; Taylor & Miller, 2001) are reduced in fishes when exposed to chronic hypoxia \( \leq 3.0 \text{ mg O}_2\text{l}^{-1} \).

Dunn & Hochachka (1986) and Dalla Via et al. (1994) observed in their studies that a metabolic reorganization takes place as a result of hypoxia that tends to follow one of two generalized patterns: (i) either the rate of anaerobic ATP production increases (Pasteur effect) or (ii) the ATP rate declines (metabolic depression).

A better understanding of ecological, behavioural, morphological, and physiological adaptations of tropical fishes was achieved by investigations of Saint-Paul & Bernardino, (1988) and Rantin et al., (1998).
D’Avanzo & Kraemer, (1994) investigated that DO fluctuations between hypoxia and hyperoxia over the diel cycle are driven by the photosynthesis and respiration cycles of phytoplankton and macroalgae in nutrient enriched coastal waters.

Hypoxia was recorded as one of the most significant causative factor in the context of health of aquatic ecosystem (Goldberg, 1995). He observed that it has caused mass fish kills and local extirpation as well as alterations in population dynamics leading to major ecological disruptions.

Diaz & Rosenberg, (1995) saw hypoxia, or low dissolved oxygen (DO), as one of the major physico-chemical factor that can potentially diminish the physiological performance of fishes in natural waters.

Almeida-Val & Hochachka, (1993) studied the biochemical adaptations of fishes exposed to hypoxic waters.

It was revealed that chronic hypoxia (days to weeks) is a common result of net respiration below the pycnocline in a density stratified water column (Pearl et al., 1998).

Besides air breathing fish species, several investigations have been undertaken on other teleosts as well, including carps, the sister group of catfishes. An interesting phenomenon in response to low oxygen concentrations has been recorded in few carps as an adaptive mechanism. Rapp Py-Daniel (2000) suggested that these mechanisms are related to the encapsulation of their swim bladder.

Chabot and Dutil, (1999); Pichavant et al., (2000); Pichavant et al., (2001); Zhou et al., (2001) studied the effects of chronic (weeks of)
hypoxia on food intake. Whereas effect of hypoxia on reproduction has been studied by Wu et al. (2003).

Due to the common occurrence of oxygen limitations in aquatic environments, many species have evolved a diversity of unique behavioral, physiological and biochemical adaptations to cope with periods of oxygen limitation.

**Enzyme Assay**

Hypoxia, or low dissolved oxygen (DO), is one physico-chemical factor that can potentially diminish the physiological performance of fishes.

Shaklee *et al.*, (1977); and Driedzic *et al.*, (1985) studied that there is no change in glycolytic enzyme activity in tissues of some other species which was proved to be wrong by Hochachka & Somero (1973, 1984). They proposed that ectothermic organisms, particularly fish, use adaptive biochemical strategies to obtain metabolic homeostasis during fluctuations in dissolved oxygen, in temperature and in some other physicochemical parameters of water.

Investigations undertaken on the fish by workers of water like Wilson (1977), Graves and Somero (1982), Panepucci *et al.* (1984, 1987), Copes and Somero (1990) revealed that LDH in fishes is usually encoded by three loci, one expressed principally in skeletal muscle (LDH-A), another in heart muscle (LDH-B) and a third one in the eye (LDH-C).

Schwantes and Schwantes (1982a,b), Farias and Almeida-Val (1992) and Lin and Somero (1995a,b) revealed by their studies on soluble form of MDH that it catalyzes the reversible oxidation of malate to
oxalacetate requiring NAD+ as a cofactor. It is involved in gluconeogenesis and lipogenesis, and in the malate-aspartate shuttle during aerobic glycolysis. The mitochondrial form (mMDH) acts in the Krebs cycle (Zink & Shaw, 1968).

Shaklee et al. (1977) found significant differences in enzymatic activity for liver LDH and white muscle MDH in fishes acclimated to different oxygen concentrations.

Wilson (1977); Graves & Somero (1982); Panepucciet al. (1984, 1987); Coppes & Somero (1990) studied the effect of hypoxia on specific properties of lactate dehydrogenases (LDH) and Shaklee et al., (1977); Schwantes & Schwantes, (1982a, b); Farias & Almeida-Val, (1992); Lin & Somero, (1995a, b) studied the soluble form of malate dehydrogenases (cMDH).


Hochachka (1980) investigated that metabolic rate reduction (metabolic depression) occur as a key feature enabling hypoxic survival in fish and other hypoxia tolerant animals.

Jhonston and Bernard (1982) studied the ultrastructure and metabolism of skeletal muscle fibers in the tench (Tinca tinca) as the effects of long-term acclimation to hypoxia. They recorded higher rates of oxygen consumption at low oxygen concentrations.

The oxygen consumption and swimming performance in hypoxia-acclimated rainbow trout *Salmo gairneri* was studied by Bushnell *et al.*, (1984). It was observed that reduced muscle enzyme activity relates to decreased locomotion during hypoxia.

Increase in the specific activities of glycolytic enzymes in different fish species exposed hypoxia was observed by Dickson and Graham, (1986); Lushchak *et al.*, (1998); Zhou *et al.*, (2000) and Kraemer and Schulte (2004). Hypoxia stress promoted the capacity of fish tissues for anaerobic energy production, all of which were suggestive of anaerobic stress.

The heart and brain show comparatively lesser degree of metabolic imbalance in response to acute environmental hypoxic pressure in trout. Dunn and Hochachka (1986) studied the metabolic responses of trout *Salmo gairdneri* to acute hypoxia.

Van den Thillart & van Waarde, (1985) suggested that during severe hypoxia aerobic metabolism cannot meet tissue energy requirements and many fishes respond by increasing energy production via anaerobic carbohydrate metabolism. In some species, the increase in anaerobic metabolism has been observed to be correlated with enzymatic changes, suggesting a reorganization of tissue metabolic capacity. Greaney *et al.* (1980) studied on *Fundulus heteroclitus* (L.), exposed to hypoxia leads to an increase in the activity of lactate dehydrogenase (LDH), the terminal enzyme of glycolysis. A shift in the tissue composition of LDH has also been noticed investigated in certain Amazonian cichlids (Almeida-Val *et al.* 1995). Extensive work
undertaken by Almeida-Val and others has revealed that hypoxia leads to a shift in the tissue composition of LDH isozymes.

The causes, consequences and compensations of environmental perturbations of oxygen transport in teleost was studied in depth by Jensen et al. (1993) and told that the efficiency of oxygen extraction or demands for energy production by tissues decreased in response to hypoxia in fish.

Almeida-Val et al. (1993) studied the important role of LDH to overcome the effects of hypoxia. Metabolic adjustments to extremely variable environments have been described as a complement of such phenotypic plasticity, particularly when fishes are exposed to hypoxia, a common event in Amazon water bodies (Val and Almeida-Val, 1995; Almeida-Val et al., 1995). The isozymic tissue distribution of LDH represents species’ adaptive tolerance to hypoxia and is adjustable to hypoxia exposure (Almeida-Val et al., 1995; 1999).

Pellitier et al., (1993) had seen the effects of hypoxia growth rate, temperature, season, and body size on glycolytic enzymes activities in the white muscle of Atlantic cod (Gadus morhua).

Investigations to study the effect of hypoxia at cellular levels saw its beginning in the last decade of last century and significant findings were observed

Relationship between up or down regulation of certain genes and the intensity of hypoxia constraint and the ability of the model to tolerate this stress was understood for the first time by Hochachka (1996). According to his review, a series of messengers (first and second) are
activated by an oxygen sensing mechanism that will affect several 100 nuclear genes and 13 mitochondrial genes when the cells are exposed to moderate hypoxia. However, the exposure to severe hypoxia will down regulate most DNA sites, inducing a decrease in mitochondrial volume densities, a decrease in Krebs cycle enzyme rates, and an increase in the ratios of anaerobic/aerobic pathways.

The change in affinity of certain enzymes, involved in aerobic and anaerobic metabolism as a result of enzymatic adaptations to hypoxia, in the long has been recorded by Lushchak et al., (1997) and Panepucci et al., (1998, 2000).

Decreased growth rate in *F. heteroclitus* was observed in response to hypoxia (Chabot & Dutil 1999, Steirhoff et al., 2003).

Virani and Rees, (2000) studied that overall metabolism (aerobic+anaerobic components) is lower during exposure to hypoxia than normoxia.

The effects of hypoxia on movements and behaviour of selected estuarine organisms was studied by Wannamaker & Rice (2000). These workers found the hypoxic fish to be less active than normoxic fish.

The aquatic organisms who do not have the option to escape from low oxygen concentrations adapt other mechanisms Boutilier, (2001). For these species, the major metabolic exercise is to decrease the capacity for ATP production via oxidative phosphorylation, leaving animals to rely on anaerobic glycolysis and/or fermentation to maintain energy equilibrium (referred to as the Pasteur effect).
However the heart and brain are affected less when exposed to hypoxia and the rate of glucose utilization by the teleost brain is thought to be limited by hexokinase activity alone (Soengas and Aldegunde, 2002).

It was found that longer-lasting hypoxia can create inhospitable habitats for fish and sedentary animals (Wu, 2002). UN Global Environment Outlook Year Book, (2003) published a paper on severe oxygen depletion of coastal waters which has significant negative consequences for economically important fisheries, ecosystems and biodiversity. These problems become even more serious if large areas are affected by hypoxia for an extended time, as fish may not be able to leave these areas.

Webester (2003) observed that the down and up regulation of glycolytic enzyme genes is caused during hypoxia.

Solld et al (2003) investigated crucian carp *Carassius carassius* gill and observed that the capacity to extract oxygen from hypoxic waters increases during chronic hypoxic exposure, through adjustments in ventilation, oxygen transport, or gill surface area.

Seymour (2006) and Steinhausen et al. (2008) studied that cardio respiratory adjustments are a prerequisite for vital functions (e.g. locomotion, digestion) and for appropriate responses to environmental changes.

Role of enzyme AChE came to scene very late but very significantly Pfeifer et al., (2005) revealed that the enzyme AChE is responsible for hydrolyzing the neurotransmittors acetylcholine into
choline and acetic acid. The enzyme controls ionic currents in excitable membranes and plays an essential role in nerve conduction processes at the neuromuscular junction. AChE is necessary for biochemical function of the brain because it is a neurotransmitter and its reduction may impair the neurotransmission. The inhibition of enzyme activity when reaches 60-90% leads to death of different fish species. Reduction in the AChE activity definitely causes physiological and behavioural modifications that reduce survival ability of the fish. Recently association of this neurotransmitter with carotid labyrinth and neuro-secretory cells of the gill has been established using immune histochemical techniques. Its role as a bio messenger to the brain in episodes of low oxygen has been postulated strongly. Transmission of change in environment through small molecule of neurotransmitters gained importance gradually in the physiology of fish and neuro-endocrine axis was revealed on the basis of many investigations on Vertebrates.

According to Gabriel and George (2005), transamination is one principal pathway for synthesis and deamination of amino acids, enabling carbohydrate and protein metabolism during fluctuating energy demands of the organism under various adaptive conditions.

Recently, they have been extended to be forecasting models for chronic hypoxia for long term tissue-specific changes. For this reason, some authors confirmed that more researches are required concerning the effect of chronic hypoxia at sub lethal exposure level to the subtropical catfishes. Therefore, the attention was shifted from natural water to simulating nature like conditions in laboratory and studies the physiological processes on experimental fish. Lot of such work came to light in various fish species out of which air-breathing fish species served
as an ideal model. A lot of investigation was undertaken to observe *in vitro* the hypoxia affects at various levels on enzyme activities especially on the brain and muscle tissues of an aquatic and well-established organism.

It was categorically accepted that enzyme analysis of organs such as muscles, brain, liver, and heart in fish can provide important information about the internal environment of the organism (Boeger *et al.*, 2003). Enzyme activities affect various chemical and biological reactions in the body of the fish ultimately resulting into major changes in genotype, phenotype and population structure etc. This revelation led to a number of investigations on fishes of varied respiratory habits using experimentally provoked hypoxic condition in the laboratory. A number of studies on many species have shown that fishes frequently respond to a change in environmental oxygen levels with changes within hematological parameters and alteration in physiological responses.

Dreidzic & Hochachka (1975) have explored the possibility that the low rate of lactate accumulation in the carp white muscle during hypoxia could be explained by the existence of alternative anaerobic pathways to glycolysis in this tissue as occurs in the muscles of numerous facultative anaerobes and in diving mammals (Magnum & van Winkle, 1973; Hochachka, Owen, Allen & Whit tow, 1975). In these animals, during anaerobiosis, carbohydrates and amino acids are utilised simultaneously with the production of a variety of end products (Hochachka, 1975). The coupling of two mitochondrial energy yielding reactions to glycolysis allows both an increase in the high energy phosphate equivalents and the maintenance of redox balance within the cell (Hochachka, Fields & Mustafa, 1973; Hochachka, 1975). However,
no evidence has been found for the accumulation of multiple anaerobic 
end products in the white muscle of either the mirror (Driedzic & 
Hochachka, 1975) or crucian carp (Johnston, 1975a).

Hochachka and Randall (1978) first suggested that long term 
responses to low-oxygen environment involves the suppression of 
oxidative metabolism in fishes of the Amazon which was later supported 
by Driedzic and Almeida-Val (1995) and West et al. (1999). However, 
Almeida-Val et al., (1999a) studied the immediate responses of hypoxia 
presented by fish of the Amazon, scarcely studied from the evolutionary 
point of view.

Mommsen et al. (1991) investigated that teleost liver contains 
distinct cell populations that differ from one another in their glycolytic 
and gluconeogenic capacities, a functional separation that would have 
been lost during homogenization.

studied that glycolytic activation with glycogen or glucose as the 
substrates and lactate as intermediate product.

Webster et al. (1994), studied on cardiac myocytes; Keitzmann et 
on aquatic turtle hepatocytes studied in isolated cell models and suggest 
that some DNA sites are suppressed and some are activated when cells 
are exposed to hypoxia.
Val, (1995) have studied aquatic surface respiration in *Colossoma macropomum* and inferred that increase in their incursions occurs to the surface when oxygen decreases in the water, and the efficiency of such innate behaviour is sufficient to allow blood oxygen efficiency.

Almeida-Val *et al.*, (1999b) studied Juveniles of *Astronotus ocellatus*, a cichlid fish that, when adult, tolerates anoxia during 6 hours at 28°C, are able to tolerate hypoxia exposure indefinitely if they are allowed to practice aquatic surface respiration, but are not able to tolerate long-term hypoxia exposure if denied access to the water surface.

**Protein Metabolism**

The effects of starvation and hypoxia upon protein turnover in red and white myotomal muscle of rainbow trout, *Salmo gairdneri* was studied by Loughna and Goldspink (1984) and was observed that when low or negative growth loss skeletal muscle protein was probably due to catabolism of protein to meet energy demands.

Boutilier *et al.*, (1988) reported that organisms are required to rely on endogenous energy reserves during chronic periods of hypoxia, and maintaining metabolic rate would seemingly lead to some degree of substrate limitation, and therefore set a temporal constraint on the individual’s survival. The other potential strategy, and perhaps the most effective, is to minimize the hypoxic insult by conforming to environmental oxygen concentrations through a significant reduction in metabolic rate.
The protein synthesis is one of the major energy consuming processes, accounting for 18-26% of cellular energy expenditure (Hawkins, 1991). But Guppy et al., (1994) observed that the down regulation of protein turnover is one of major contributing factors to the depression in ATP turnover and metabolic depression at the whole animal level.

The Amazon fish under experimental conditions were shown to be either activating anaerobic metabolism or suppress oxidative metabolism when exposed to hypoxia (Almeida-Val et al., 1993).

In 1995, Almeida-Val and co-workers investigated that the characteristics already described in fish, particularly tropical species and viewed that, several options of morphological and anatomical adaptations are linked to metabolic adjustments.

Jibb and Richards, (2008) investigated that depressing metabolic rate during hypoxia is a key mechanism for the conservation of endogenous substrates thereby extending the amount of time that can be spent under oxygen limiting conditions. This process, however, requires coordinated metabolic reorganization aimed at decreasing ATP demands to match the capacity for production.

In comparison to this exhaustive account of work to study the effect of stressor like hypoxia on fishes of different types of waters abroad, the scenario in India has not been very encouraging. In spite of having a rich diversity of climate, water and fish with reference to different respiratory habits, the investigations on Indian fishes are very few.
Since the study of biochemical adaptations comprehending enzymatic activity and changes in intermediary metabolites together with haematological parameters seems appropriate to understand the changes involved during the events of hypoxia, the present work aimed at explaining how this three parameters are influenced by hypoxia.