Man induced changes in the aquatic environment result in chronic stress that have negative effects on aquatic life. Most of the loss of freshwater biodiversity originates from systemic failure to understand the linkages between human activities and their impact upon freshwater ecosystems. The aquatic environment is definitely more abused than the terrestrial, as many industrial and domestic wastes are dumped directly into streams, rivers, lakes and oceans. Fishes are an integral part of most aquatic ecosystems and degradation of the aquatic environment contributes to a multifold depletion of fish stocks.

2.1 Water Quality studies

2.1.1 Physico-chemical aspects

River pollution has been quite alarming in recent years as a result of waste discharges from industries, sewage outfall from townships, pilgrimage centres, etc. Water quality criteria are developed on the basis of scientific information about the effects of pollutants upon a specific use of water. The criteria, therefore, are defined as the acceptable levels of concentration of pollutants for a particular use and describe the water quality requirements for protecting and maintaining the aquatic biota. Investigations on water resource involving the quality, biotic and abiotic aspects, along with the anthropogenic factors have widely been carried out (Stormer et al., 1996; Nevo et al., 2001).

Most of the Indian rivers are polluted by industrial effluents, agricultural runoff and sewage discharge. The degree of pollution is generally assessed by studying the
physical and chemical characteristics of the water body. Pollution results in the degradation of physico-chemical as well as biological quality of water. Therefore, it is important that potentially impacted aquatic ecosystems be regularly monitored for physico-chemical and biotic integrity in view of protecting them. Biological investigation enumerates the flora and fauna of the water body. Changes in water quality exerts a selective action on the flora and fauna which constitute the living population of water and the effects produced in them can be used to establish biological indices of water quality. This can be related to all forms of life, from microbes to mammals. The interrelationships between various physico-chemical and biological characteristics helped in understanding the nature of the intricate interactions occurring in this ecosystem.

The majority of Indian rivers have been studied extensively for physico-chemical aspects by several authors (George et al., 1966; Ray and David, 1966; Ajmal et al., 1985; Shukla et al., 1989). All these studies revealed that the indiscriminate discharge of effluents, agricultural runoff, sewage, domestic wastes etc., into the rivers deteriorated the quality of the water thereby affecting the biotic and abiotic components. Dhanapakiam et al. (1999) analyzed the water quality of River Cauvery and pointed out that the water supply from the river source was not fit for industrial and domestic purposes and cannot serve as scarcity alternative of drinking water in summer. Jameel and Hussain (2003) investigated the seriousness of the discharge of untreated sewage into the Uyyakondan channel water, a tributary of River Cauvery, by analyzing the physico-chemical and bacteriological parameters and observed that the sewage water not only deteriorated the quality of channel water but also is a major source of pollution in the river.

The ever-increasing population and expansion of the Guwahati city have induced immense pressure on the rivers and drains leading to qualitative change in water. Das et al. (2003) revealed extreme pollution in Bharalu river with very low level of dissolved oxygen, high load of BOD, COD, phosphate and ammoniacal nitrogen which affect the quality of water and jeopardizing the survival of aquatic life. The domestic wastes, municipal sewage, various effluents and agricultural runoff have
deteriorated the quality of Godavari river water with high fluctuation in all the physico-chemical parameters especially with raised values of alkalinity, hardness, COD and chloride indicating the pollution of riverine ecosystem (Sanap et al., 2006).

Seasonal variation had a significant role in determining the water quality parameters in rivers. Suvarna and Somashekar (1997) assessed water quality of the river Cauvery and its tributaries Arkavathi and Vrishabhavathi using Bhargava’s water quality index and revealed that the water was moderately alkaline with dominant sodium and bicarbonate ions and also observed variation in turbidity and pH along with an increase in phosphate during the period of high flow. Similar study was carried out by Jagtap et al. (2002) in Purna river and noticed variation in the different ions which might be attributed by seepage of ground water from aquifer or due to the dilution of water during monsoon seasons.

Altun et al. (2003) analyzed water quality parameters in River Degirmendere and Galyan River with respect to seasonal changes and compared with various standards. Sharma and Gupta (2004) made an attempt to study the water quality of Kunah-Khad stream in Himachal Pradesh and revealed that water in the stream is highly polluted during monsoons with high turbidity and BOD which is beyond the permissible limit, but the levels of dissolved oxygen, pH, hardness, chloride and alkalinity were within the permissible limit. Bhasker et al. (2003) observed a remarkable seasonal variation in the waters of the river Torsa with high alkalinity and high load of faecal bacteria.

The water quality characteristics of Hathali stream to quantify the pollution status with respect to seasonal changes were carried out by Sharma and Gupta (2004) and found variations in dissolved oxygen and BOD level during summer which reflected the high organic pollution. The drastic variation in the physico-chemical parameters of Hatmur reservoir water was also observed by Rajput et al. (2004) with the change in seasons. Shrivastava (2005) noticed seasonal variations in the water quality characteristics in Chambal command area of Kali Sindh river where the parameters exceeded the desirable limits. The quality of the water was affected by various inputs
from municipal and industrial effluent and non point sources such as runoff and agricultural waste.

The comparative picture of both physico-chemical and biological score of the Damodar river revealed a certain degree of perturbation occurred in water quality due to the discharge of wastewater. The dominance of calcium and bicarbonate ions in the surface water and the presence of sodium ions in the sub surface waters indicated high levels of total dissolved solids (TDS) which affect the quality of river water thereby affecting the life of organisms (Ganguly et al., 1999; Singh, 2002). A similar deterioration of water quality was observed in the short stretches of the River Brahmani by Mitra (1999) and Muduli et al. (2006).

Most of the physico-chemical parameters of the surface water of River Beas in Shivalik hills were found to be within the permissible limits of drinking water standards, except the high coliform count in all the samples indicating the faecal pollution (Sharma, 2004). Water quality studies of Yamuna river at Mathura also indicated high pollution with high turbidity, total hardness, total dissolved solids and the presence of metals such as chromium, nickel, cadmium, lead and zinc beyond the tolerance limits (Singh and Gupta, 2004). Hydro-geochemical analysis of Bhadra river was carried out by Vijayakumar et al. (2005) and noted the dominance of calcium and bicarbonate ions in surface and sub surface waters, but total dissolved solids of sub surface water is much higher than the surface water.

Many studies have been carried out to evaluate the impact of human activities on different ecosystem. Madhyastha et al. (1999) studied the impact of anthropogenic activities on the physico-chemical and microbial characteristics of a few riverine stations located in the vicinity of major temples of Dakshina Kannada district in Karnataka State. They observed high organic pollution load in this water at all times. The main cause of deterioration of water quality was the lack of proper sanitation, unprotected river sites and increasing anthropogenic activities.

Hussain and Ahmad (2002) observed the variations of water quality parameters and the presence of heavy metals in the bed sediment of River Pachin for the three major
flow periods. The study exposed that the variability in the physico-chemical parameters for different flow periods might be assigned to dilution of river water by direct run off, human activities and organic load. Ecological studies of Banjara lake with reference to physico-chemical characteristics and water pollution have been carried by Swarnalatha and Rao (1998) and found chlorides, calcium and magnesium levels within the permissible limits whereas the levels of total solids and total hardness were exceeding the WHO (1983) and ISI (1983) limits indicating the impacts of sewage and domestic waste.

Bellandur lake is one of the major lakes of Bangalore city and is subjected to severe pollution stress from the urban community. Impact of urbanization on Bellandur lake has been investigated by Chandrasekhar et al. (2003). The addition of effluents from urbanized Bangalore city has changed the characteristics of the lake from being a natural ecologically healthy lake to an artificial reservoir of domestic sewage and industrial effluents. DO and COD values were above the permissible limits and it was reported that if the present conditions continue for long the lake may soon became an ecologically inactive water body. Sunkand and Patil (2004) noted that the water quality of Fort Lake of Belgaum (Karnataka) is of inferior quality and not suitable for drinking purposes. Thilaga et al. (2005) observed that Ooty lake has been polluted mainly due to discharge of municipal sewage and surrounding runoff resulted in the eutrophication with large quantities of nutrients.

Studies conducted in the water bodies of Kerala indicated the deterioration of quality of water. Unnithan et al. (1974) reported the organic matter pollution in Cochin backwaters. They found that accumulation of pollutants beyond the tolerance level affected the survival of species of various fauna and consequent reduction in total number of species. The industrial effluents to the Periyar river had a great influence in the degradation of water quality. There are reports that during monsoon the concentration of nutrients is quite high in the bottom resulting in eutrophication which leads to algal blooms and consequent increase in BOD (Joseph et al., 1984). They also noted that during summer the river experienced high temperature, high chloride and phosphate content, low pH and low dissolved oxygen whereas during
monsoon low temperature, low chloride and phosphate, high pH and high dissolved oxygen. Variations in water temperature of several rivers in Kerala have been reported. In Kallada river the variation was between 23.5°C to 33°C, in Neyyar it ranged between 24°C to 32°C (Madhusoodanan, 1992).

Koshy and Nair (1999 & 2000) noted considerable variation in the physico-chemical and biological parameters of River Pamba indicative to pollution. The deterioration of the river may be due to the flow of sewage, domestic waste and faecal matter especially during the Sabarimala pilgrimage season. The surface water of the Neyyar river in Kerala evaluated for a period of one year has shown an inverse relationship between BOD and DO, CO₂ with pH, and also found to have a close relationship of COD with chloride and conductivity (Prasannakumari et al., 2003). They also observed that chloride and turbidity were high during monsoon and the nutrients were abundant during monsoon and post-monsoon seasons.

The ecological status of Chitrapuzha river, one of the tidal tributaries of Periyar river, receiving effluents from several industrial establishments was evaluated with respect to species composition, community structure and residual pollutants in plant and animal tissues. Dominance of tolerant species of flora and fauna, low population density and high residual concentration of heavy metals indicated the existence of stress condition in the area (Remani et al., 2004).

Harilal et al. (2004) investigated hydrogeochemistry of two rivers of Kerala, Karamana and Neyyar with special reference to drinking water quality. The various physico-chemical and biological characteristics of water samples were compared with the prescribed standards and it was found that all the parameters except BOD and total coliform were within the limit of potability, whereas an increase was noticed with the lower reaches of rivers and might be due to the anthropogenic activities and input of waste into the rivers. In 2006, Harikumar and Madhavan studied the water quality and associated environmental issues in relation to Kerala inland waterways. They reported that the domestic sewage, market waste and excessive weed growth led to the pollution of water bodies of Kerala.
2.1.2 Bacteriological aspects

The bacteriological examination of water is used to indicate the possible deleterious effects of water quality on human health. The water quality monitoring of major rivers indicates that organic pollution is predominant and almost all the surface water sources are contaminated to some extent by coliform group of bacteria that make them unfit for human consumption. Sewage contains many human enteric pathogens, which are frequently transmitted by ingestion or by contact with polluted water (Piper et al., 1982). Faecal coliforms and *Streptococcus* are accepted indicators of potential health hazards. The survival of indicator bacteria in aquatic ecosystems is a complex phenomenon regulated by a great variety of factors, including water temperature and predation (Anderson et al., 1989). Microbiological pollution produced as a consequence of the sewage discharge may be studied by the analysis of microorganisms, the densities of which are quantitatively related to potential hazards to human health (Aiki and Nishio, 2006).

Oyun river in North central Nigeria was evaluated by Adewoye and Lateef (2004) and observed a high microbial load and faecal contamination in the water samples and they also found that the industrial effluent discharged into the river enhanced the growth of microbial population. Pathogenic bacteria of man such as *Staphylococcus*, *Klebsiella*, *Bacillus*, *Enterobacter*, *Escherichia*, *Streptococcus* and *Pseudomonas* were also isolated from the water samples.

Several authors reported the presence of high coliform levels exceeding the standard limits in the rivers indicating the high level of organic pollution (Somasekhar, 1984 and Unni, 2000). The bacterial number enhanced from December to May in most of the rivers and declined in June due to rain. They also noted an increase in the coliform bacteria during festival season. Biological investigations of river Umshyrpi, a major river in Meghalaya, carried out by Rajurkar et al. (2003) revealed that the biological parameters such as total coliforms and faecal coliform bacteria exceeded the permissible limit, which indicated the unpotability of water. The increase in number of total coliform and faecal coliform clearly showed the inflow of waste due
to rapid growth in the residential and the commercial activities in the catchment area and also the existence of dry latrines along the river. Radha and Seenayya (2004) have observed a good correlation in the distribution of heterotrophic bacteria with suspended solids during the monsoon seasons and inferred that the physico-chemical parameters of water had a great influence in the distribution of bacteria in the Hussain Sagar lake.

The enviro-ecological status of Mandakini river was assessed during religious festival to know whether the domestic wastes from the pilgrim settlements or the mass bathing degrade the quality of water and has been reported that the bacteriological count was very high which is unfit for domestic purpose (Rekha et al., 2004). Another study on the microbiological analysis of bore-wells was carried by Bahadoor et al. (2004) during different seasons and found that bacterial population was high in the monsoon compared to other seasons whereas faecal coliform bacteria were detected during monsoon and winter. Beegum et al. (2003) evaluated the coliform count of different water resources and observed that the coliform count was highest in river water followed by well, supply water and tube well. They also revealed that season had a significant influence on variation of coliform counts and the count increased from pre-monsoon to monsoon and decreased gradually in post-monsoon, which further reduced during winter. Rekha et al. (2005) evaluated the microbiological quality of drinking water at Satna and noted high microbial load. A periodic analysis of two dam waters in Nanded district, Maharashtra by Surve et al. (2006) indicated monthly variation in the population of *Citrobacter* and *E. coli*.

### 2.2 Water quality and stress on fishes

Water quality is the most important factor for fish health. Fishes can tolerate the adverse effects of aquatic stressors to some extent by physiological mechanisms. Stress in fact is the effect of any environmental alteration or force that extends homeostatic or stabilizing processes beyond the normal limits, at any level of biological organizations (Esch and Hazen, 1978). Stress response of fish is analogous
in many ways to that occurring in the higher vertebrates (Peters et al., 1988; Chen et al., 2000).

Rivers are under stress and in danger because of potential pollution and contamination risks due to anthropogenic effects. This eventually leads to chronic stress effects on the inhabitants. Various aspects of stress in fish fauna have been reviewed by Love (1980); Wedemeyer et al. (1976); Mazeaud et al. (1977) and at ecosystem level by Esch and Hazen (1978). If an animal cannot adapt, the stress will be lethal, but even if less severe, it will still predispose to disease if pathogens are present (Bullock and Snieszko, 1971; Portz et al., 2006). The effects of water quality on fishes vary considerably with species, life stage and previous exposure to stress (Pickering, 1981; Wedemeyer et al., 1996). Fishes may incur additional energetic costs associated with stress responses from physical and chemical fluctuations in aquatic systems (Barton and Iwama, 1991). Poor water quality can prompt the reallocation of energy from secondary physiological processes towards primary processes like metabolism, immune function etc. Thus, adequate or preferably “optimal”, water quality is essential for fish to lead a healthy life. Short-term exposure to poor water quality can result in permanent damage or mortality if physical or chemical variables are allowed to reach lethal levels and synergize in a deleterious manner (Carmichael et al., 1984; Robertson et al., 1987; Erickson et al., 1997; Pavlidis et al., 2003).

2.2.1 Physico-chemical stress on fishes

**Temperature** affects the physical and chemical properties of water and all chemical reaction equilibria. Water temperature influences the gas solubility and therefore the dissolved gas content in water. Fishes are poikilothermic, so their surrounding water temperature is critical to their physiological rates and metabolic processes. Any fluctuation in the temperature may affect the growth, reproduction and survival of fishes (Dippner, 1997; O’ Brien et al., 2000; Nordie, 2006). As body temperature increases, rate of biochemical reactions generally increase and affect the membrane transport flux dynamics (Elliot, 1981; Alabaster and Lloyd, 1982; Neill and Bryan,
1991; Heath, 1995; Ham et al., 1997). Thermal stress occurs when the water temperature exceeds the optimal temperature range, thus initiating changes that disturb normal physiological function resulting in energy expended towards stress responses, and even a potential decrease in individual survivorship (Brett, 1958; Fry, 1971; Elliot, 1981). Most fishes can acclimate to gradual temperature changes over months, such as with season. However, rapid water temperature changes result in thermal stress at lethal conditions. Lipid reserves, plasma proteins, blood osmolality and serum electrolytes may decrease due to ion and osmoregulatory dysfunction creating physiological imbalances with thermal stress (Houston and Schrapp, 1994; Swanson et al., 1996; Claireaux and Audet, 2000). The thermal stress affects other water quality constituents such as dissolved oxygen, carbon dioxide content etc., and total dissolved solid concentration in water (Hettler, 1976; Claireaux et al., 1995).

**The Dissolved oxygen** is the most critical and limiting water quality variable (Fry 1947, 1971), because water is the respiratory medium of fish. The oxygen consumption rate and the degree of respiratory stress are dependent upon the species, life stage, body weight and activity levels (Alabaster and Lloyd, 1982). Respiratory stress involves increased gill ventilation frequency and haematological changes (Lochmiller et al., 1989). It also exhibits altered behavioural patterns such as erratic swimming, side lying and sometimes stunted or retarded growth.

**pH** has direct effect on other water quality variables and consequently on fishes. Acidic pH (4.2-5.0) acts directly on gill membranes, increasing ion permeability generally causing a loss of sodium and chloride ions. This change in membrane ion permeability causes decreased osmotic pressure and increased haematocrit, plasma protein concentration and blood viscosity (Wood and McDonald, 1990; Randall, 1991). High pH also causes increased blood pH, decreased gill membrane potential, gill corrosion and increase in the influx of sodium and chloride.

Variation in the haematological parameters was reported in *Labeo rohitha* (Hamilton- Buchanan) fingerlings exposed to sub lethal acidic and alkaline pH (Dutta et al., 2004). At acidic pH, fishes showed elevated levels of haemoglobin,
haematocrit level, plasma cortisol, glucose, cholesterol and lactic acid content. The study also revealed that the severity of alterations in the tissue and blood parameters were greater in the acidic pH than at alkaline pH indicating that the fishes were under greater physiological stress at the acidic pH.

**Nitrite and nitrate** in higher concentrations induce a significant reduction in the haematocrit value in the juveniles of *Labeo rohitha* (Subhendu et al., 2005) irrespective of duration of exposure. They also revealed that the strength of erythrocytes cell membrane was decreased due to stress.

**Pollution** had a direct effect on the distribution of fishes. According to the survey by Saravanan et al. (2003) a decline of fish species was noted in the polluted sites of Cauvery river. Stressful situations caused by external factors (pollutants, diet, physical condition) can lead to increased susceptibility, reduced rate of survival, growth and reproductive success in several fish species (Sakai et al., 1998; Adams et al., 2001; Luckenbach et al., 2003). Therefore, there is a need to characterize the normal biochemical patterns that would indicate stress in healthy animals under optimal conditions. Smart (1981) related the water quality inducing stress in intensive fish culture. Poor water quality or even fluctuations in water quality affects appetite, growth and food conversion efficiency. He also explained the influence of environmental oxygen, ammonia and carbon dioxide concentrations on fish performance.

As fish fauna serves as a food source it is important to study the impact of water pollution on these organisms. Any change in the natural conditions of aquatic medium, whether physical, chemical and biological, causes several physiological adjustments in fish (Black, 1958; Arkoosh et al., 1998). The physiological and biochemical changes in fishes exposed to pollution stress have been recorded (Gill and Pant, 1981; Amudha et al., 2002; Baruah et al., 2004). The qualitative and quantitative profile of biochemical constituents (Protein, Carbohydrate and Lipid) are influenced by a variety of environmental factors. Seasonal and geographic changes also have a significant effect on biochemical composition (Zenebe et al., 1998).
Lipid peroxidation has been established as a major mechanism of cellular injury in plants and animals and it is used as an indicator of oxidative stress in cells and tissues (Girotti, 1998; Winston and Di Giulio, 1991). Disrupted tissues are known to undergo lipid peroxidation at a faster rate than the normal ones; therefore it can be used as a measure of oxidative damage (Nielsen *et al.*, 2000; Agarwal and Kale, 2001). Oxidative stress has become an important aspect of toxicology and has received increasing attention. It has ecological significance, particularly in the aquatic environment, which is the ultimate sink for many pollutants capable of producing oxidative stress. Lipid peroxidation is not only considered as a measure of oxidative stress, but of stress in general and thus serve as an indicator of fish health. Fishes can serve as valuable bioindicators and as alternative models for understanding oxidative stress (Kelley *et al.*, 1998; Sole *et al.*, 2004). Fish tissues contain large quantities of polyunsaturated fatty acids (PUFA) which are essential for membrane function. However, PUFAs are highly susceptible to oxidative attack (Hsieh and Kinsella, 1989; Guderley and St. Pierre, 2002). Higher lipid peroxidation level has been reported in stored tissues of fishes exposed to low temperature (Ingemansson *et al.*, 1995; Stephan *et al.*, 1995). According to Witas *et al.* (1984) the lipid peroxidation activity differs among species and is tissue-specific.

The concentration of malondialdehyde (MDA), a marker of lipid peroxidation changes, was measured in the different muscle segments of male and female common tench, *Tinca tinca* (L.). The malondialdehyde content varied in different body segments, but the highest MDA values were observed in the cranial segment for both sexes. Its localization in close proximity of the gill cavity, gills and the arteries distributing oxygenated blood was one of the prerequisites for increased oxygen saturation (Halamickova *et al.*, 2003).

The common carp and Nile tilapia exposed to pesticides indicated that fish developed tissue specific adaptive responses to protect cells against oxidative stress, with gill showing the highest levels of change in superoxide dismutase (SOD) activity compared to kidney and brain and also showed that common carp is more sensitive than Nile tilapia (Ozcan *et al.*, 2004). European eels exposed to harbour water
developed tissue specific peroxidative damage in gill, kidney, and liver; with the gills being most affected (Ahmad et al., 2004). Freshwater cyprinids from polluted areas showed signs of oxidative stress, with higher SOD and catalase (CAT) activities and stronger lipid peroxidation when compared to fish from unpolluted areas (Gul et al., 2004).

Environmental stress invokes compensatory metabolic changes in the organs of an animal through modification and modulation of the quantity and quality of proteins. Proteins are one of the most important and complete group of biological materials comprising of the nitrogenous constituent of the body and performing different biological functions (Lehninger, 1984; Kapil and Ragothaman, 1999; Muushigeri, 2003). They are also involved in major physiological events to maintain the homeostasis of the cell. Therefore, the assessment of the protein content can be considered as a diagnostic tool to determine the physiological processes of the cell.

Saravanan et al. (2003) highlighted the alterations in protein content in two fishes collected from polluted and non-polluted sites of River Cauvery. They found the depletion of protein content in muscle and it could be due to mobilization of protein from muscle to blood, to compensate to certain acidosis caused by the lactate accumulation. They also revealed that fishes from polluted sites had more depletion of protein content in muscle than non-polluted areas.

Carbohydrates are essential components of all living organisms and form a major class of biological molecules. According to Clarke (1975) carbohydrate is considered to be the first among the organic nutrients degraded in response to stress conditions imposed on animals. The freshwater fishes, Oreochromis mossambicus and Mystus montanus from the sewage and polluted stations of River Cauvery, showed a reduction in the carbohydrate content of muscle tissue and this might be attributed to its utilization for the increased energy demand posed by the pollution stress (Saravanan et al., 2003).

Environmental stress induces variations in blood tissues of fish. The haematological characters of three Cyprinid fishes, according to the species, gender, seasonal
differences and the water quality effects were carried out by Orun et al. (2003). The blood parameters showed a significant difference between warm months and cold seasons and also observed a high level of leucocytes, neutrophil and monocyte in female fish especially in the reproductive season, but the levels of erythrocytes, haemoglobin and haematocrit level were high in males and there was no gender effect on erythrocyte indices. A significant difference in haematological and biochemical parameters was found in *Clarias gariepinus* with the change in temperature (Adeyemo et al., 2003).

According to Fernandez and Mazon (2003), haematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fish lives could exert some influence on some haematological characteristics. Therefore stress can induce changes in the haematology of an organism. So, fish haematology is considered as an essential tool for the biologists as a frontline sensitive indicator of vital physiological and biochemical functions as well as status of nutrition, health, diseases and stress in response to changing environmental conditions. Blood parameters are known to be moderately sensitive biomarkers of acute and chronic stress (Schramm et al., 1999).

The haematological index has been employed effectively in monitoring the response of the fish to the stressors and thus its health status under such adverse conditions and reflects the ecological condition of its habitat. The haematological parameters in fish may be influenced by intrinsic factors such as sex, reproductive stage, age, size and health and they are also affected by external factors like seasonal dynamics, water temperature, environmental quality, food and stress (Mahajan and Dheer, 1979; Rios et al., 2002). The striking alterations in the blood parameters and associated pathological changes in fishes under the influence of various toxic agents have attracted the attention of the workers in the field.

Literature revealed that species difference as well as seasonal difference can cause changes in the haemoglobin content and erythrocyte count (Molnar and Tamassy, 1970). The microhaematocrit, erythrocyte numbers and haemoglobin concentrations
in carp and trout of both sexes change with respect to various temperatures, season and the nutritional status and these parameters have been used as indices of the physiological condition of fishes. Results indicate that microhaematocrit can be used as a general index of haematological status in the carp (Houston and Dewilde, 1972; Cormack, 1975).

Histopathological studies are recommended for making more reliable assessments of biochemical responses in fish exposed to a variety of environmental stressors. The gills being delicate structures get affected easily if the surrounding media is contaminated (Holcton and Randall, 1969; Roy and Munshi, 1991). The gill epithelium is the site of gas exchange, ionic regulation, acid-base balance and nitrogenous waste excretion by fishes. They reported the rupture in the tip of the primary lamellae of *Cirrhinus mrigala* exposed to toxicants and this may be due to the epithelial swelling and coagulation of mucous layer around the epithelium. Evans (1987) reported that various environmental pollutants have been found to affect the morphology of the gill epithelium. Fukuda (1983) also observed oedema and hyperplasia in goldfish gills after exposure to linear alkyl benzene sulfonate. The gill of mature *Channa punctatus* exposed to effluent of industrial wastes in the Cauvery river water was found to have apparent changes in the gill structure such as swelling of lamellae, alteration of epithelium, hyperplasia and shrinkage of blood capillaries (Dhanapakiam et al., 1998).

Mallatt (1985) analyzed the effect of short-term exposure of pollutants on fish gills. He suggested that hyperplasia of squamous epithelial and chloride cells serves as a physical and physiological defence mechanism against the toxicants and pointed out that toxicant-induced histopathological gill changes are not specific for any class of chemicals and could be regarded as a pathophysiological adaptation.

The pathological changes in the liver tissue of various freshwater fishes under toxic stress have been studied (Anitha and Tilak, 2003; Tilak et al., 2005). All these studies revealed that stress-induced degeneration of cytoplasm in hepatocytes.
Atrophy, formation of vacuoles, and rupture in blood vessels, necrosis and sometimes the disappearance of hepatocytic cell wall were also observed

### 2.2.2 Bacterial stress on fishes

Unlike higher vertebrates, fishes are less immuno-competent, which is the major factor predisposing them to innumerable disease outbreaks. A disease breaks out when a susceptible fish is exposed to a virulent pathogen under unfavourable environmental circumstances. The incidence of a disease is thus the result of a complex interaction between the fish, the disease agent and the aquatic environment (Snieszko, 1975). Usually a disease manifests when the fish is subjected to a shift in its physiological condition like infection by a pathogen in the spawning time or due to an external stressor. Such a stressed fish population becomes vulnerable to a potential pathogen either from the environment or a carrier fish. Many carp farms have been wiped out due to Epizootic Ulcerative Syndrome (EUS) (Miyazaki and Egusa, 1972). *Aeromonas* bacteria are widely distributed in aquatic environments (Palumbo et al., 1992; Chang and Nog, 2004) and cause bacterial haemorrhagic septicaemia and epizootic ulcerative syndrome in numerous freshwater (Shao et al., 2004) and marine species (Lilley et al., 1997).

Stress is not always the deciding factor in triggering infection; some bacterial strains like *Aeromonas salmonicida* and *Mycobacterium marinum* are primary pathogens that infect even with little stress while other species like *A. hydrophila*, invade only heavily stressed fish (McCarthy and Roberts, 1980; Doukas et al., 1998). Most of the bacterial pathogens are capable of independent existence outside fish except a few are obligatory species. *A. hydrophila* may also become an opportunistic invader infecting fish under stressful conditions or in concert with other pathogens. Ulcerous dermatitis on fish caused by *A. hydrophila* is one of the useful biomarkers of polluted or other stressful environment (Sindermann, 1988). Thus an opportunistic pathogen, *A. hydrophila* is associated with clinical conditions in a wide variety of poikilothermic and homeothermic hosts (Elwan et al., 1985).
Many strains of pathogens like *A. hydrophila* exacerbate diseases such as spring viremia of carp or *Saprolegnia declina* infection on salmonids, myxobacterial and other protozoan infections on the larval branchial cavity (Inglis et al., 1993). *Aeromonas hydrophila* infects a wide range of animals, including mammals, and is a common cause of disease in warm water fish culture throughout the world (Thune et al., 1993; Austin and Adams, 1996; Yu et al., 2004). In Southeast Asia, fish kills due to *A. hydrophila* contribute a substantial economic loss to the fish farming industry (Llobrera and Gacutan, 1987; Thampuran et al., 1995).

*A. hydrophila* along with other pathogens and stress conditions cause haemorrhagic septicaemia (Leung et al., 1995). Incidence of a disease suppresses the immune response, enhancing the susceptibility of infection to an alarming rate (Yono et al., 1991; Fevolden et al., 1993, 2003). Unfavourable environmental factors such as crowding, low-dissolved oxygen and higher organic content, industrial pollution, temperature fluctuation, physical injuries and physiological conditions like spawning may also pave the way for *A. hydrophila* infection (Pippy and Hare, 1969; Shotts et al., 1972). Intensive culture system and poor physiological and environmental conditions make the fish susceptible to diseases (Couch, 1975). Though fishes possess functional defence systems, blood cells are less immuno-competent which is a major factor predisposing them to innumerable disease outbreaks (Snieszko, 1974). In tropical countries, monsoon brings an abundant supply of pesticides from the adjoining agricultural fields, which along with a fall in ambient temperature makes the riverine fish vulnerable to diseases like EUS (Braaten and Hektoen, 1991) that lead to the mass mortality of fishes (Haines, 1983). Among the fish pathogen *A. hydrophila* is reported to be the gravest and a major economic problem in warmwater fishes (Amin et al., 1985; Rungapan et al., 1986).

*A. hydrophila* infection causes symptoms such as haemorrhagic septicaemia characterized by the presence of external surface lesions, local haemorrhages in the gills and vent, abdominal distension and internal symptoms like anaemia, accumulation of ascitic fluid and damage to the internal organs like kidney and liver (Jhingran and Das, 1990). Besides *A. hydrophila*, other identified pathogens and
their preferred hosts are *Vibrio anguillarum* on eel (Ullrich, 1992), *A. salmonicida* on salmon (Wiklund and Dalsgaard, 1998) and the fungus, *Aphanomyces*, on carp (Callinan et al., 1995). *A. hydrophila* when injected into healthy *Etroplus suratensis* and *O. striatus* induced dermonecrotic lesions resembling EUS (Panthiratne et al., 1994). *Cyprinus carpio, Clarias batrachus, Osphronemus* sp. when experimentally infected with *A. hydrophila* at three different concentrations of 10³, 10⁵, and 10⁷ cells; of the three, *Clarias batrachus* was found to be the most susceptible (Supriyadi, 1986).

Chen et al. (2000) and Itano et al. (2006) studied the effect of *Nocardia* sp. infection on yellowtail, *Seriola quinqueradiata* by different treatments (intraperitoneal injection, ingestion, immersion, co-habitation and intradermal injection). They found that the fish challenged by intraperitoneal injection died within 10 days and showed white nodules and histopathological changes, whereas mortalities of fish infected with intradermal injections were low, the immersion challenge system closely resembles natural exposure and is more appropriate than the injection challenge method. Ramalingam and Ramarani (2006) have indicated that the facultative and Gram-negative cum heterotrophic bacterial forms are pathogenic to fish population and prawn species. Ichthyofauna richness has been reported to be a sensitive indicator of relative condition of rivers and lakes, so reduction in number of fish species is clearly observed in places where aquatic pollution is severe.

*Yersinia ruckeri* is the causative agent of yersiniosis or enteric redmouth disease leading to significant economic loss in salmonid aquaculture worldwide and the infection may result in a septicaemic condition with haemorrhages on the body surface and the internal organs. Hunter et al. (1980) and Tobback et al. (2007) reported that the carrier fishes of *Y. ruckeri* were found to transmit the pathogen to healthy fishes under stressful situation of higher temperatures.

*Flavobacterium psychrophilum* can also cause disease in non-salmonid fish. This has been associated with systemic disease in eel, *Anguilla anguilla* (L.) and three species of cyprinids, common carp, *Cyprinus carpio* L., crusian carp, *Carassius carassius*
(L.), and tench, *Tinca tinca* (L.), in Europe (Lehmann et al., 1989; Austin & Austin, 1999). Fish also showed signs of anaemia, exophthalmia, haemorrhage on the gills, necrotic myositis, necrotic scleritis and cephalic osteochondritis (Ekmann and Norrgren, 2003). The effect of water temperature on the progress of infection and the course of the disease under experimental conditions was considerable (Holt, 1989; Lorenzen, 1994; Nematollahi et al., 2003).

*Lactococcus garvieae* is a Gram-positive coccal pathogen, which is capable of infecting both warm water marine fish species such as yellowtail in Japan (Kitao et al., 1981) and cold freshwater species like rainbow trout in Northern Italy (Eldar et al., 1996) and in grey mullet with congestion and haemorrhage of the internal organs. *Streptococcus* related diseases occur in many cultured marine and freshwater fish and it affects *Oreochromis* sp. and *Sarotherodon* sp., as well as grey mullets, *Mugil cephalus* and silver carp, but not in common carp (Kitao et al., 1981; Hubbert, 1989; Eldar et al., 1994). Vibriosis is a stress mediated disease due to *Vibrio parahemolyticus* and occurs spontaneously in various fishes, sometimes leading to septicaemia, which is fatal (Sakata and Hattori, 1988). *Aeromonas* and *Pseudomonas* sp. and unidentified Gram-negative bacteria were reported to be the cause of high mortalities in reared snakehead, *Ophiocephalus striatus* and catfish, *Clarias batrachus* (Lio-Po et al., 1998).

### 2.2.2.1 Haematological effects

Haematological studies are useful in assessing the health status of fish when subjected to adverse conditions (Munkittrick and Leatherland, 1983). The changes in erythrocytes and leucocytes of stressed fish have been extensively investigated. Several workers have documented the changes in haematological parameters of air-breathing fishes like *Mystus vittatus* (Banerjee, 1989) and *Channa punctatus* (Mahajan and Dheer, 1979) during monsoon period; the stress in the form of pollutants though not results in gross changes and mortality leads to changes in haematological profile. The first line defence to any stress is a rise in the WBC level (Harikrishnan et al., 2003). Studies on RBC counts, haemoglobin content and
haematocrit concentration in brook trout and rainbow trout infected with *A. hydrophila* have been reported (Neville, 1979). Anaemia associated with *A. hydrophila* infection is adduced to the ability of these organisms to produce protease enzymes that can haemolyse erythrocytes. This confirms the observations on the incidence of decreased erythrocyte count with experimentally induced infection in *Cyprinus carpio* (Harikrishnan *et al*., 2003) or in goldfish, *Carassius auratus* (Brenden and Huizinga, 1986b). In response to an infection, changes in differential leucocyte counts also reflect fish health; viral, bacterial, and fungal infections lead to the decrease in lymphocyte frequency and an increase in the frequency of granulocytes (Rehulka, 1996).

A decline in RBC count, haematocrit and haemoglobin content associated with symptoms of severe anaemia was recorded by Waagbo *et al.* (1988) in Atlantic salmon with Hitra diseases. The haematological analysis clearly indicated severe anaemia and this was caused by loss of blood cells, probably from haemorrhaging. Similar decline in RBC, haematocrit and haemoglobin level combined with signs of anaemia was also described by Hoffman and Lommel (1984) in cases of proliferative kidney diseases. The non-significant difference in MCV, MCH, and MCHC between the diseased and healthy fish testifies the fact that anaemia was due to blood losses from skin lesions. These blood indices indicated active erythropoiesis to compensate for the loss.

Many authors have described anaemic states in cases of bacterial infection of salmonids. The diseased fish had a significantly lower red blood cell count and lower haematocrit and haemoglobin levels. Haematological and biochemical analyses carried out in blood and organs of juvenile Atlantic salmon, *Salmo salar* L., infected with cold water vibriosis showed that they were anaemic and indicated haemorrhages (Pippy and Hare, 1969). Harbell *et al.* (1979) also recorded the same in Coho salmon, *Onchorhynchus kisutch* infected with a highly virulent *Vibrio anguillarum*. Cardwell and Smith (1971) found a prominent effect on haematocrit and haemoglobin in juvenile Chinook salmon with vibriosis. For a severe *Aeromonas* infection in Atlantic salmon, Foda (1973) reported a decrease in haemoglobin.
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Amend and Smith (1975) demonstrated a reduction in RBC, haematocrit and haemoglobin level in Infectious Haematopoietic Necrosis (INH) virus-infected rainbow trout. The infected fish had reduced corpuscular counts, MCH and MCHC and increased immature erythrocytes, but the percentage of leucocytes remain unchanged, while in the differential count, the percentage of lymphocyte was found significantly increased and neutrophils decreased. The relative percentage of increase in lymphocyte was probably due to the absolute reduction in neutrophils.

Bacterial infection with *Aeromonas* and *Streptococcus* in rainbow trout, *Salmo gairdneri* resulted in major fragile erythrocytes and raised the ESR values but treatment with antibacterial agents improved the values of both parameters (Barham *et al.*, 1980). Active *Aeromonas salmonicida* infections significantly depressed the leucocrit; however, no change was noted during the incubation phase prior to the development of an epizootic (Wedemeyer *et al.*, 1983). Brenden and Huizinga (1986a) observed drastic changes in haematological parameters in mice inoculated with the virulent fish strain of *Aeromonas hydrophila* during the first 36 hours of infection. Innoculation led to septicaemia and tissue damage followed by death and they suggested that the endotoxin of the bacteria *Aeromonas* were contributing the pathogenesis in mice.

Literature revealed that *Cyprinus carpio* infected with *A. hydrophila* had a marked decrease in RBC counts, haematocrit and haemoglobin, plasma protein with resultant anaemia (Takahashi, 1984) whereas *Salmo gairdneri* infected with *A. hydrophila* showed an elevated haematocrit, plasma glucose level and increased WBC counts (Peters *et al.*, 1988). The rainbow trout, *Salmo gairdneri* infected with *A. salmonicida salmonicida* showed decreased haematocrit level, haemoglobin content, erythrocyte, lymphocyte and thrombocyte counts and increased neutrophil and monocyte counts indicated a strong phagocytic activity, however, no morphological changes were observed (Lehmann *et al.*, 1989).

The physiological and haematological changes in Chum Salmon artificially infected with Erythrocytic Necrosis Virus had lower plasma glucose and blood lactic acid and
there was considerable variation in the haematological parameters too, such as lower RBC counts, haematocrit and haemoglobin concentrations, higher WBC counts and less fragile erythrocytes. The haematological data indicated that erythrocytes of infected fish had higher MCV, depressed MCHC and slightly lower corpuscular haemoglobin. The infected fish also showed erythrocytic inclusions in the cytoplasm of RBC (Kirk, 1974; Haney et al., 1992). The haematological observation on a hill stream fish Garra gotyla gotyla (Gray) showed comparatively higher haematological values than other freshwater teleosts of the same size (Rautham & Aggarwal, 1996). Changes in WBC and differential counts are important in the assessment of the state of health of *Clarias gariepinus* where leucopenia and leucocytosis have been reported under exposure to pathogens (Van Vuren et al., 1994; Omoregile and Oyebanji, 2002).

Mastan and Qureshi (2002) highlighted the haematological investigations of EUS affected fishes of Bhopal such as *Puntius sarana, Mastacembelus armatus, Macrognathus aculeatus* and *Mystus cavasinus*. EUS affected fishes showed a substantial decline in the number of erythrocytes, lymphocytes and haemoglobin content, but there was an increase in the number of granulocytes and monocyte. The increase in the number of granulocytes in diseased fishes may be due to the increase in the tissue damage by the pathogens and other stress factors. The decline in the lymphocyte may also be the effect of toxin from the bacteria. Rehulka (2003) examined the changes in the blood of juvenile rainbow trout, *Onchorhynchus mykiss* during the occurrence of a natural infection of viral haemorrhagic septicaemia virus (VHS).

A remarkable variation in RBC, haemoglobin, haematocrit, MCV, MCH & MCHC and histological changes such as fusiform and spherical shaped nucleus and echinoid cell membrane were observed in Black Goby (*Gobius niger* L.) collected from a site where petroleum refinery is located (Selma and Hatice, 2004). The Nile tilapia (*Oreochromis niloticus*), infected with *Edwardsiella tarda*, an aetiological agent of several diseases of freshwater fish was studied by Karasu and Yildiz (2004). The remarkable reduction in haematocrit, haemoglobin concentration, erythrocyte count
and total plasma protein with an increase in the total leucocyte number and plasma glucose was observed in the diseased fish. The post-mortem examination also showed discoloration of gills, haemorrhages of the skin and cutaneous ulcerations. Increase in leucocyte count during infection clearly indicated the defence of the fish against the pathogens.

Benli and Yildiz (2004) have attempted to assess the changes in blood parameters following spontaneous infection of tilapia, *Oreochromis niloticus* in order to evaluate the severity of the disease on the basis of blood alterations. There were signs of haemorrhages of the skin and cutaneous ulcerations in the mouth. The blood parameters showed a significant reduction in haematocrit, haemoglobin content, erythrocyte count and total plasma protein in infected tilapia and an increase was observed in the total leucocyte count and plasma glucose level.

Another interesting work was done by Yildiz and Bekcan (2005) on some blood parameters in the eel (*Anguilla anguilla*) infected with *A. hydrophila*. They observed significant reduction in haematocrit value and total plasma protein, but an increase in the plasma glucose and potassium levels and also that haemopoeisis was found severely affected by the bacterial infection. Jose *et al.* (2006) evaluated the effects of an experimental challenge with *Mycobacterium marinum* on the blood parameters of Nile tilapia, *Oreochromis niloticus*. The fishes were inoculated intraperitoneally with $10^8$ cfu/ml of *Mycobacterium marinum* and blood samples were analyzed for a period of 84 days. The inoculated fishes showed significant difference in the haematological parameters such as RBC, MCV, MCH, MCHC and WBC which characterized an acute infection. The decrease in erythrocyte count and the haematocrit could suggest a tendency to develop hypochromic microcytic anaemia.

The changes in the basic haematological indices may also be induced by some parasitic protozoa, or certain metazoan parasites. Kawatsu (1978) found that the RBC, Hb, Hct and MCH declined with the growth of *Eudiplozoon nipponicum*. Similar studies in *Labeo calbasu* infected by *Trypanosoma* also showed considerable changes in the haematological parameters in both the sexes as compared to healthy
It was found that ESR, small lymphocytes, monocyte and eosinophils increased, while RBC, PCV, MCV, haemoglobin, clotting time, specific gravity and plasma viscosity etc., decreased considerably (Saxena and Shukla, 2006).

2.2.2.2 Biochemical effects

The variation in biochemical parameters can be used as a diagnostic tool in the evaluation and assessment of pathological state of fish and the water quality (Isamah et al., 2000). Literature revealed that the freshwater fishes infected with the bacterial species such as *Aeromonas* sp., *Pseudomonas aeruginosa* and *Edwardsiella* sp. induce changes in the biochemical parameters (Ayala, 1987; Hsiao and Chen, 1987; Anderson et al., 1989; Brady and Lasso, 1992; Qureshi et al., 2000 and Jayashree, 2004).

Toxic cyanobacterial cells containing microcystin induce oxidative stress in exposed tilapia fish, *Oreochromis mossambicus* under laboratory condition. Cyanobacterial cells induced antioxidant defences and increased the lipid peroxidation level to a greater extent than the non-crushed cells. The liver was the most affected organ followed by kidney and gills (Fatima et al., 2000; Jose et al., 2005). *Heteropneustes fossilis* when exposed to microcystis extract resulted in increased lipid peroxidation indicative of oxidative stress in primary hepatocytes (Gupta and Guha, 2006). The Yellow perch, *Perca flavescens*, were assessed for oxidative stress on parasitism by the nematode *Raphidascaris acus* and metacercariae of the digenean *Apophallus brevis*. Fish infected with *R. acus* at the contaminated site tended to have higher levels of lipid peroxidation than the uninfected fish at the same site and those fishes infected with metacercariae expressed higher levels of lipid peroxidation. This supported the use of lipid peroxidation as a biomarker of water contamination as well as for the pathological effects by parasitism. Marcogliese et al. (2005) clearly indicated that contaminants and parasites occurring together exacerbate oxidative stress in fish.

The freshwater giant prawn, *Macrobrachium rosenbergii* inoculated with *Pseudomonas aeruginosa* MTCC1688 revealed pathogenic symptoms in some tissue
biochemical parameters. The infected prawn showed profound changes in the total protein content of the body muscle and hepatopancreas. The decrease in total protein indicated the proteolysis in tissues and this may be attributed to the action of extracellular secretion of the proteases by the bacterial strain. The chitinoclastic nature of the opportunistic bacterial pathogen revealed that the diseases in shrimps is the outcome of a pathogenic chain linkage involving environment (medium)-bacterial population-shrimp population (Ramalingam and Ramarani, 2006).

Biochemical analyses carried out from the organs of *Salmo salar* L. infected with cold water vibriosis indicated reduced levels of total protein and the effect of *Aeromonas* as casual agents of severe skin lesions in salmonids (Harbel *et al*., 1979; Rehulka, 2002). Atlantic salmon, *Salmo salar* L. infected by *Vibrio salmonicida* showed significantly reduced total protein content, albumin, triglycerides and cholesterol level (Waagbo *et al*., 1988). They also found an elevated level of branched chain amino acids leucine, isoleucine and valine in muscle extracts of diseased fish. The liver is the major site of oxidation of essential amino acids, except for the branched chain amino acids which are catabolized primarily in muscle tissues. Waagbo *et al.* (1988) also found a significant increase in certain aminoacids like valine, leucine and isoleucine in muscle extracts of infected fish with *Aeromonas*, whereas serine level showed a reduction.

The carbohydrate metabolism of the flounder, *Paralichthys olivaceus*, was altered by the *Edwardsiella* infection. The plasma glucose level was significantly lower in the infected fish whereas the tissue glycogen level did not change significantly. The protein metabolism also changed in infected fish (Miwa and Mano, 2000; Levesque *et al*., 2002). Barham *et al.* (1980) found that an infectious disease may affect the glucose level in fishes. They also found lower level of glucose concentration in infected rainbow trout with bacterial aetiology. However, Haider (1969) reported Viral Hemorrhagic Septicaemia (VHS)-induced hyperglycemia in the course of a nephritic syndrome. A 50% fall in the plasma glucose level in fish suffering from anaemia resulted in the depletion of liver glycogen due to the failure of liver
functions. An increase in glucose level found in infected fish is characteristic of a stress response (Black et al., 1961; Harbell et al., 1979; Winston, 1991).

Fishes are susceptible to parasite infestations and are detrimental to fish health. There are only a few studies on how parasitic infestations may affect antioxidant mechanisms in fish, but all of them reported alterations in oxidative status and antioxidant defences of parasitized fish. Infection by the trematode Clinostomum detruncatum did not induce significant changes in the SOD and CAT activities in the muscle of freshwater silver catfish (*Rhamdia quelen*), but lipid peroxidation activity significantly increased, which indicated that parasitic infection induced oxidative stress and higher level of membrane damage in the fish muscle (Bello et al., 2000). Infection of common carp by the cestode Ptychobothrium sp. showed increased antioxidant enzyme activities in liver and head kidney (Dautremepeuixs et al., 2003). The antioxidant response is dependent on the fish species and on the parasite. It has been suggested that antioxidant activities of the host can be modulated through the functioning of the parasitic life cycle (Buchanan and Lindenstrom, 2002).

### 2.2.2.3 Histopathological effects

Histopathology is a well established tool to assess the qualitative changes of the affected organs and the patterns of recovery. In fishes, extensive studies are available reporting the organ-specific pathological changes. It was reported that, fishes like *Clarias batrachus*, *Salmo gairdneiri*, and *Ictalurus punctatus* experimentally infected with *A. hydrophila* resulted in necrosis and haemorrhage in the kidney, liver, pancreas and intestine (Weinreb, 1958; Ventura and Grizzle, 1988; Angka, 1990; Candan, 1990). Donta and Haddow (1978) have pointed out that *A. hydrophila* toxins are cytotoxic. Release of bacterial toxins cause acute haemorrhage and necrosis of vital organs (primarily liver and kidney), leading to rapid death due to organ failure (Huizinga et al., 1979). Generally gill is one of the main sites of antigen uptake and retention. In fish, liver is considered to be the main scavenging organ where endothelial cells and macrophages are highly endocytic towards self and non-self substance that are to be eliminated from the circulation (Noya et al., 1995).
Astronotus ocellatus, an ornamental fish infected with Motile Aeromonas Septicaemia (MAS), contained large amount of red ascitic fluid accumulated in the abdominal cavity along with haemorrhages in the liver and kidney (Soltani et al., 1998). The eel, Anguilla japonica, infected with A. hydrophila showed necrosis in the muscle bundles with septic haemorrhage, spleen damage, fatty liver, renal hematopoietic tissue atrophy and necrosis in nephron (Chein and Chein, 1994).

The histopathological investigations have been proved to be a sensitive tool to detect direct toxic effects of chemical compounds within target organs of fish in laboratory experiments and in field investigations (Wester and Canton, 1991; Schwaiger et al., 1992, Schwaiger, 2001). In addition to assessing toxic effects, a further aim of these studies was to observe the severity of changes in the organs which may occur from environmental stressors other than contaminants (Teh et al., 1997) and which are known to occur as a consequence of adverse environmental conditions (Snieszko, 1974; Anderson, 1990). These investigations helped to differentiate between organ lesions induced by diseases and other environmental factors.

Stress-related pathogenesis has been reported by various authors. The histopathological studies on fishes infected with different bacterial species indicated a clear damage to the digestive tract and a subsequent cellular reaction. Bacterial proliferation could be the cause of this digestive damage (Crespo et al., 2001; Bobadilla et al., 2007). A comparative study conducted on blood chemistry and histopathology of Nile tilapia (Oreochromis niloticus) infected with Vibrio vulnificus or Streptococcus iniae, revealed significant variations in the blood parameters as well as moderate histological changes in gill, trunk, kidney, liver, spleen etc. (Chen et al., 2004).

Tencalla and Dietrich (1997) reported the effect of Microcystis in rainbow trout, Onchorhynchus mykiss. The typical chord structure of trout liver disappeared and cytoplasm of hepatocytes was condensed. The hepatocyte nuclei began to condense and cytoplasm of these cells was highly vacuolated. Fischer and Dietrich (2000) also pointed out the effects of microcystin in the kidney and hepatopancreas of Cyprinus
carpio which damage renal proximal tubular cells and hepatocytes within few hours. Carbis et al. (1997) found that freshwater fishes, *Heteropneustes fossilis* and *Cyprinus carpio* exposed to microcystin had showed degenerated gills with necrosis, epithelial ballooning, folded lamellar tips and exfoliation of the lamellar epithelium. *Heteropneustes fossilis* after short term and long term treatment with microcystin clearly revealed the damages in the liver and hepatocytes after 3 hours and at a later stage the hepatocytes become swollen and the cytoplasm become granular, whereas the gills showed structural modifications (Gupta and Guha, 2006). The microcystin reached the gill through blood circulation and resulted in the enlargement of the epithelial cells, the mucous cells and the chloride cells during the initial period and in later stages proliferation of the chloride cells were noticed.

The Japanese flounder *Paralichthys olivaceus* infected with *Edwardsiella tarda* causes hypertrophy of the liver. The size of the liver cells and nuclei of the challenged fish were apparently larger than the controls (Miwa and Mano, 2000). Swelling of the liver was also reported in the Japanese eel, *Anguilla japonica* infected with *Edwardsiella tarda* (Miyazaki and Kaige, 1985). Macroscopic and histopathological changes were observed in cultured turbot, *Scophthalmus maximus* (L.) infected with *A. salmonicida* (Bjornsdottir et al., 2005) and *Edwardsiella tarda* (Padros et al. 2006). The changes include haemorrhages, necrosis and degeneration in skin and muscle, haemorrhages and necrosis in kidney, degeneration in the heart muscle and fusion of secondary gill lamellae, hypertrophy and hyperplasia in mucous, chloride and epithelial cells.

A comparative study of the two fishes, cod (*Gadus morhua* L.) and halibut (*Hippoglossus hippoglossus* L.) showed pathological changes in muscle, gill and haemorrhages in liver following infection with the Gram-negative bacterium, *Mortella viscose* (Gudmundsdottir et al., 2006). They also revealed that cod is more sensitive than halibut to infection with *M. viscose* and also found that the bacterium had wide host specificity.