Review of Literature

The success of ornamental fish culture depends on the health status of the candidate species (Lipton, 2006). Being aquatic, and secondarily being forced to remain under crowded conditions, the ornamental fishes are subjected to different diseases of varying nature (Bright and Sreedharan, 2009). Bacterial infections are considered as the major cause for diseases and mortality (Grisez and Ollevier, 1995). A complete understanding of the aetiological agent, the pathogenesis, antigenicity, epizootiology and the inter-relationship of stress-related and environmental factors is essential for successful management and control.

2.1 Water quality and fish diseases

The water easily spread most of the pathogens. It is necessary to have an understanding of water quality in order to successfully diagnose and correct aquarium diseases. Stress has been linked as the primary contributing factor of fish disease and mortality in aquaculture (Petric et al., 2006). Several biotic and abiotic factors influence the growth of fish (Jobling, 1996). Among the various physical factors affecting the aquatic environment, temperature is of paramount importance and is considered as the ‘abiotic master factor’ for fishes (Brett, 1971). Global climate change is suggested to potentially affect freshwater fisheries by lowering productivity in wild fish populations and in intensive aquaculture systems worldwide (Ficke et al., 2007). As fishes are poikilotherms, drastic change in their surrounding water temperature will influence their metabolic processes, behavior, migration, growth, reproduction, and survival (Fry, 1971; Portner, 2001). Researchers are making continuous efforts to define thermal tolerance of various fish species of aquaculture.
importance (Rishikesh et al., 2009). Long-term changes in the environmental temperature induce ectothermic animals to display compensatory responses (which include changes in the metabolic enzymes and tissue chemistry) that are suggested to mitigate the effect of temperature on metabolism (Hazel and Prosser, 1974; Hochachka and Somero, 1971).

Temperature beyond the optimum limits of a particular species, however, adversely affects fish health by increasing metabolic rate and subsequent oxygen demand, invasiveness and virulence of bacteria and other pathogens which in turn may cause a variety of pathophysiological disturbances in the host (Wedemeyer et al., 1999). Temperature affects virtually all biochemical, physiological activities of fishes. The survival and growth of poikilothermal teleosts are immediately influenced by temperature fluctuations in their environments. All teleost species have developed their own specific adaptive mechanism, both behavioural and physiological, to cope up with temperature fluctuations (Prosser and Heath, 1991). These adaptive capabilities enable them to survive through acclimation and adaptation to stressful temperature conditions (Hazel and Prosser, 1974). Identifying the range of temperatures tolerated by a species is important to determine the viability of its growth. Up to a species-specific maximum, fish growth rates will accelerate with increasing temperature, after which they sharply decline (Fielder et al., 2005; Jobling, 1996). Temperature beyond optimum limits of a particular species adversely affects the health of aquatic animal by increasing metabolic rates and subsequent oxygen demand (Chatterjee et al., 2004).

Crowding is a factor involved in physiological stress (Barton, 2002). The increase in stocking densities can alter the immunological responses and physiological processes, mainly those related to metabolism and behavior (Vijayan et al., 1990;
Irwin et al., 1999; Barcellos et al., 2004; Kristiansen et al., 2004 and Schram et al., 2006). It has been noticed that inappropriate stocking densities can alter lipid metabolism, mainly of triglycerides, in brook charr, *Salvelinus fontinalis* (Vijayan et al., 1990). In gilthead sea bream, *Sparus aurata*, different stocking densities altered fatty acid metabolism, with a decrease in hepatic oleic acid, a monounsaturated fatty acid important as energy source, mainly in higher stocking densities (Montero et al., 1999). Also, crowding is responsible for the increase in plasma cortisol, which plays an important role in the low efficiency of immunological responses under these conditions (Mommsen et al., 1999; Di Marco et al., 2008).

Water pH affects metabolism and physiology of fish. Alkaline pH 7 to 8 is highly suitable for better growth of fish. Robert and William (1986) found that in channel catfish, excretion of ammonia at pH 6 increased; whereas, it decreased with increased pH. Saha et al. (2002) and Scott et al. (2005) indicated that ammonia excretion increased with increasing pH (alkalinity), while growth decreased.

Dissolved oxygen is the most important and critical parameter, requiring continuous monitoring in ornamental fish culture systems. This is due to the fact that fish aerobic metabolism requires dissolved oxygen (Timmons et al., 2001). Optimum level of dissolved oxygen recommended is 4 to 5 mg/l for warm water fishes (Wedemeyer and Goodyear, 1984). Studies of freshwater teleosts indicated that low dissolved oxygen concentrations also can modify juvenile and adult growth rates, feeding rates, habitat use and susceptibility to predation, as well as adult reproductive activities (Magnuson et al., 1985; Suthers and Gee, 1986; US-EPA, 1986; Kramer, 1987; Poulin et al., 1987; Saint-Paul and Soares, 1987).

The physiological activities of fish are also subjected to changes in environmental factors such as dissolved $O_2$ level (Jordan and Steffensen, 2007; *Studies on Pathophysiology of fresh water ornamental fishes..., Ph. D. Thesis, 2012*).
A recent study found that environmental temperature had profound effects on the metabolic competition mode of southern catfish (*Silurus meridionalis* Chen), possibly due to the increased oxygen demand and decreased availability of environmental dissolved oxygen at high temperatures (Pang *et al*., 2010). Conformers, such as the Adriatic sturgeon (*Acipenser naccarii*), cannot maintain their resting O$_2$ consumption rate during hypoxia, and it will decrease linearly with decreasing dissolved O$_2$ content (McKenzie *et al*., 2007). Hypoxia can cause physiological stress and cellular damage as well as inhibit repair mechanisms (Jones, 1985). Spot and pinfish can detect a variety of DO concentrations but they do not necessarily avoid hypoxia (Wannamaker and Rice, 2000). Wannamaker and Rice (2000) suggested that these fishes may have relatively lower physiological costs when occupying hypoxic areas. Shultz *et al.* (2011) recommended that the dissolved O$_2$ concentrations during holding of bonefish in the context of live-release angling tournaments do not deviate from that of ambient sea water, which was typically 6 mg/l. Similar studies on live-release bass tournaments have recommended to the anglers and tournament organizers to monitor DO concentrations so as to maintain their required levels of dissolved O$_2$ for recovery (Suski *et al*., 2006; Furimsky *et al*., 2003; Suski *et al*., 2003).

Ammonia is the principal nitrogenous waste product of fishes that represents 60% to 80% of nitrogenous excretion of fish (Handy and Poxton, 1993; Salin and Williot, 1991). It is also, the main nitrogenous waste material excreted by gills beside urea and amines and an end product of the protein catabolism (De Croux *et al*., 2004). Among all the water quality parameters, which affect fish, ammonia is considered as one of the most important after oxygen (Francis – Floyd and Watson, 1996). Under intensive rearing conditions, and particularly when effluent is reused, ammonia...
concentrations may reach levels that limit fish survival and growth (Haywood, 1983). Ammonia can cause reductions in growth or even death (EPA, 1998; Meade, 1985; Salin, and Williot, 1991). In water, total ammonia consists of non toxic (ionized ammonia) referred to as ammonium (NH$_4^+$) and toxic un-ionized ammonia (NH$_3$). The equilibrium between these two forms is dependant on the pH and temperatures. Ammonia is measured as total ammonia nitrogen (TAN) which represents the sum of NH$_4^+$ and NH$_3$. The NH$_3$ molecule is soluble in lipids which is 300 to 400 times more toxic than NH$_4^+$ (Haywood, 1983; Thurston et al, 1981). Un-ionized ammonia (UIA-N) can readily diffuse across the gill membranes due to its lipid solubility and lack of charge (Aysel and Koksal, 2005). When ammonia accumulates to toxic levels, fish cannot extract energy from feed and will fall into a coma and die (Hargreaves and Tucker, 2004).

Ammonia tends to block oxygen transfer from the gills to the blood and can cause both immediate and long term gill damage (Joel and Amajuoyi, 2010). Also it can cause impairment of cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish, crustaceans and mollusks (Smart, 1978). Chronic un-ionized ammonia exposure may affect fish and other organisms in several ways, e.g. gill hyperplasia, muscle depolarization, hyper excitability, convulsions and finally death (Ip et al., 2001). Toxicity of ammonia to fish has been intensively investigated in numerous fish species (Aysel and Koksal, 2005; El-Shafai et al., 2004; Lamarie et al., 2004). The acute and chronic toxicities of ammonia have been reviewed for fresh water species (Tomasso, 1994; Handy and Poxton, 1993; Russo and Thurston, 1991; Haywood, 1983; Ruffier et al., 1981).

Uncontrolled level of ammonia in culture environment may not only lead to mortality but may prevent the fish from achieving its full genetic potential in terms of...
growth and reproductive capability. At the sub-lethal value of ammonia, it can compromise the well being of fish by jeopardizing its health (Ajani, 2008). Smith and Piper (1975) and Smart (1976) found that the most characteristic feature for chronic exposure of rainbow trout to ammonia was the appearance of swollen, rounded secondary gill lamellae or telangiectatic capillaries in the secondary lamellae. Also, Kirk and Lewis (1993) reported that the gills of rainbow trout exposed to 0.1 mg/l ammonia for 2 h exhibited deformation of the lamellae. Ammonia concentrations of above 0.2 mg/l in fish ponds have a tendency to harm the fishes and is recommended that the UIA-N concentrations be maintained below 0.1 mg/l (Abdalla and Heba Allah, 2011).

Ammonia induces detrimental changes in tissue structure, cell function, blood chemistry, osmoregulation, disease resistance, growth and reproductive capacity (Jeney et al., 1992). Chronic exposure can result in the deterioration of several physiological functions any one of which may be the ultimate cause of death (Russo, 1985). Ammonia may affect gill structure (Smart, 1976), respiratory function (Chen and Lin, 1992; Knoph, 1996) and oxygen consumption (Smart, 1978) in aquatic animals. Keeping animals healthy in intensive aquaculture depends on preventing accumulation of toxic waste products such as ammonia. Ammonia concentration of 1–2mM is common in the blood of marine invertebrates (Haberfield et al., 1975). Higher concentrations are presumably toxic because they perturb acid–base balance with too much alkalinity (Hammen, 1980).

Nitrite (NO\textsubscript{2}) is a potential contaminant in aquatic environments that receive nitrogenous waste (Grosell and Jensen, 1999). Nitrite is formed from ammonia and may accumulate in aquatic systems as a result of imbalance of nitrifying bacterial activity (\textit{Nitrosomonas sp.} and \textit{Nitrobacter sp.}) (Masser et al., 1999). High levels of
nitrite in the water is a potential factor triggering stress and cause high mortality in aquatic organisms (Ferreira da Costa et al., 2004; Wang et al., 2004; Jensen, 2003; Martinez and Souza, 2002). Several studies have examined the toxicity and physiological effects of NO$_2^-$ in fish (Das et al., 2004; Knudsen and Jensen, 1997; Doblander and Lackner, 1996). When NO$_2^-$ reaches the blood, it crosses the erythrocyte membrane and oxidizes haemoglobin to methaemoglobin. The toxicity of nitrite may result from a combination of effects rather than from a simple effect, such as methaemoglobinemia, in particular.

An elevated ambient nitrite concentration is problematic for freshwater fish, as nitrite is actively taken up across the gills in competition with chloride (Eddy and Williams, 1987). The principal effect of such nitrite loading is a progressive oxidation of haemoglobin to methaemoglobin, but several other physiological changes occur (Jensen, 1990). The interference with branchial ion exchange together with methaemoglobinemia and likely tissue hypoxia suggests that major changes may arise in blood O$_2$ transport and respiratory properties resulting in perturbations of electrolyte and acid–base status (Jensen et al., 1987). Knudsen and Jensen (1997) showed that nitrite interferes with K$^+$ homeostasis in carp leading to an extracellular hyperkalaemia. Aggergaard and Jensen (2001) have shown among rainbow trout exposed to nitrite increased in plasma K$^+$ with a concomited decrease in plasma Cl$^-$. This rise in plasma K$^+$ is suggested due to the release of K$^+$ from intracellular compartments (Knudsen and Jensen, 1997). Nitrite binds competitively to haemoglobin oxidising it to metHb, a variant causing the blood to appear brown in colour (hence the name “brown blood disease”) and vastly reduce the ability to bind and transport oxygen (Jensen, 2003; Martinez and Souza, 2002; Hargreaves, 1998).
Acute and chronic effects of nitrate have been reported in several fresh water fish species (Hamlin, 2006; Camargo et al., 2005) and marine invertebrates (Kuhn et al., 2010; Romano and Zeng, 2007; Camargo et al., 2005; Hirayama, 1974). The mechanisms of nitrate toxicity to aquatic animals are due mainly to methemoglobinemia, caused by the oxidation of hemoglobin (Hb) to methemoglobin (MetHb) in blood (Camargo et al., 2005) consequently reducing oxygen binding capacity and ultimately resulting in respiro-circulatory constraints. In fish a MetHb reductase system compensates for MetHb formation by conversion of MetHb to Hb (Freeman et al., 1983). Nitrate is taken up via the branchial system in fishes. However due to the low permeability of the gills to nitrate, uptake is limited and other mechanisms are suggested (Stormer et al., 1996). Another possible pathway might be trans-dermal uptake of nitrate in the gastro intestinal tract, as was reported for nitrite in European flounder, *Platichthys flesus* (Grosell and Jensen, 2000).

### 2.2 Oxygen consumption

Metabolic rate is the most fundamental biological rate as it represents the rate of energy uptake, transformation and allocation (Brown et al., 2004). The measurement of respiration in fish has been used as a tool to estimate metabolic rates. Several different states of respiratory metabolism have been defined and categorized for fish viz., standard, routine, active and anaerobic (Cech, 1990; Brett and Groves 1979). Routine metabolism refers to the rate of metabolism that encompasses spontaneous movements (Fry, 1971). Oxygen consumption rate is one of the physiological responses that can be correlated with changes in environmental parameters, because it is related to the metabolic work and energy flow that organisms must channel to homeostatic control processes (Salvato et al., 2001).
Measurement of the oxygen consumption rate in fish is a valid method to assess the effect of environmental factors, such as temperature, salinity, exposure to pollutants, light intensity and dissolved oxygen. It allows the estimation of the energy costs associated with the physiological stress that these factors impose on organisms (Brougher et al., 2005; Altinok and Grizzle, 2003). Oxygen consumption rate is often used to examine energy utilization to determine the environmental conditions that result in maximal utilization of input energy for weight gain in an organism (Shi et al., 2011; Meade et al., 2002). Much information on the physiological responses of fish to adverse water quality has obtained from research work involving freshwater species, particularly the rainbow trout *Oncorhynchus mykiss* (Perry et al., 1982). Knowledge of an organism's metabolic rate at different temperatures is important as it provides a basic indication of its energy requirements in different environments. Temperature is the most important extrinsic controlling factor influencing metabolic rate (Fry, 1971). The metabolic theory of ecology proposed by Brown et al. (2004) persuasively demonstrates how a few simple, well-founded physical principles concerning energy and temperature can explain an impressive proportion of the natural variability in organism-level productivity, developmental rates, mortality and other life history traits. Therefore, effects of temperature on metabolic rate have received considerable attention. Body size is seen as the most significant endogenous factor affecting oxygen consumption (Armitage and Wall, 1982).

Many authors expressed temperature/oxygen consumption relationships in terms of Q_{10} values as it is generally assumed that the effect of temperature on oxygen consumption is determined by the influence of temperature on speed of biochemical reactions. Tamura (1939) reported oxygen consumption rate of *Haliotis discus hannah* (abalone) at 0.053 to 0.085 ml/g/h at 22–23 °C. Uki and Kikuchi (1975) reported the
oxygen consumption rate of *H. discus hannai* to be 0.184 to 0.504 ml/individual/h at 20 °C with 1.5 to 4.8 g body weight.

### 2.3 Ammonia excretion

The nitrogenous metabolic byproducts excreted by the fish, especially ammonia is the major water quality concern next to dissolved oxygen content (Colt and Armstrong, 1981; Handy and Poxton, 1993; Tanaka and Kadowaki, 1995) and being toxic to fish, crustaceans and molluscs can limit production in aquaculture (Epifanio and Srna, 1975; Wickins, 1976; Russo, 1985; Allan *et al*., 1990; Russo and Thurston, 1991). Total ammonia as nitrogen (TAN) is the main nitrogenous excretion product from fish and typically constitutes 80 to 90% of the total nitrogen excreted (Fivelstad *et al*., 1990; Handy and Poxton, 1993). Ammonia excretion is known to be affected by factors such as species, body weight, water temperature, feeding and ration size (Yager and Summerfelt, 1993). Quantification of ammonia excretion is important for assessing the environmental impact of culture operations (Dosdat *et al*., 1996) and for calculating loading densities in fish transport (Froese, 1988). Total ammonia nitrogen excretion rates are directly related to dietary nitrogen and protein intake in fish (Handy and Poxton, 1993). Both oxygen consumption and ammonia excretion rates are affected by animal's body size, activity, handling, diurnal rhythm, feeding and environmental conditions e.g. temperature, salinity the animal is exposed to (Saroja, 1959; Crear and Forteath, 2000).
2.4 Bacterial diseases

Bacterial disease is one of the most important diseases in ornamental fishes and a significant cause of high fish morbidity and mortality rates (Barker, 2001). It can grow to large number, invade to fish and spread the disease when reaching the suitable condition. Many stress factors could contribute to bacterial infection in ornamental fish, viz., poor water quality, crowding, transportation and inadequate nutrition (Musa et al., 2008).

There are several studies on fish bacteria identification, experimental infection or disease resistance (Azad et al., 2001; Al-Harbi and Uddin, 2004; Cai et al., 2004). A perusal of literature has revealed that the freshwater forms have been infected with the bacterial species such as *Aeromonas* sp., *Pseudomonas aeruginosa*, *Edwardsiella* sp and *Enterobacter cloacae* (Jayashree, 2004 and Qureshi et al., 2000). *Aeromonas* and *Pseudomonas* sp. have been isolated mostly from diseased and moribund fishes from intensive fish culture systems (Lipton and Lakshmanan, 1986). Ornamental fish may develop acute systemic and/or chronic granulomatous diseases, where the bacteria may cause damage to the fins and ulcerations of the skin and these infections may be caused by primary pathogens, but more commonly are due to stress induced secondary infections (Wooley et al., 2004). Clinical signs include lethargy, erythema and petechiation of the skin and fins, along with ulcerations of the skin, and internal lesions and these lesions are most commonly associated with Gram-negative organisms such as *Aeromonas*, *Pseudomonas*, *Vibrio*, *Flavobacteria*, *Yersinia*, and *Edwardsiella* spp. The pathogenic bacterial isolates of fish such as *Pseudomonas aeruginosa* and *A. hydrophila* were tested for their pathogenicity (Lipton, 1987). The author reported that the fish isolate of *P. aeruginosa* had a lethal dose of $1.5 \times 10^5$ cells / fish for *Cyprinus carpio* and $4.2 \times 10^5$ cells for *O. mossambicus*. The fish pathogen
A. hydrophila had lethal doses of $2.1 \times 10^6$, $6.8 \times 10^5$ and $3.2 \times 10^6$ cells/fish, respectively, for *C. carpio*, *L. rohita* and *O. mossambicus*.

*Aeromonas hydrophila* is a Gram-negative aerobic and facultative anaerobic, oxidase-positive motile bacterium and is also considered a normal flora as well as a primary and secondary fish pathogen, including ornamental fish (Aoki 1999; Austin and Austin, 1999). Hettiarachchi and Cheong (1994) described *A. hydrophila* as the major cause of disease in freshwater ornamental fish. Jongjareanjai et al. (2009) showed that the majority of the bacteria isolated was *A. hydrophila* (27/30) and one strain of *Enterococcus durans*, *Flavobacterium sp.* and *Serratia marcescens* from sick ornamental fish.

*Aeromonas hydrophila* possesses many factors related to its virulence, such as extracellular products including aerolysins, α and β haemolysins, enterotoxins, proteases, haemagglutinins and adhesins (Sha et al., 2002). Uma et al. (2010) detected hemolysin and aerolysin gene by PCR in an *A. hydrophila*, isolated from infected koi carp, *Cyprinus carpio*, an important ornamental fish species. In Malaysia aquarium shop, 60% of *A. hydrophila* were isolated from sick freshwater ornamental fish (Musa et al., 2008). Mosharrof Hossain (2008) isolated six strains of *Aeromonas* spp bacteria from the gourami (*Colisa lalia*) by 16S rDNA sequencing analyses that are pathogenic to freshwater fish. Among them, three were under *Aeromonas veronii* species, two were *Aeromonas* sp ATCC and one was *Aeromonas hydrophila*. According to Noga (1996), motile aeromonad infection (MAI) is likely the most common bacterial disease of fresh water fish, all of which are probably susceptible. Pathogenic *Aeromonas sobria* has been identified as causative agent of ulcerative fish disease in farmed European perch (Goldschmidt et al., 2008). The outbreak of a
disease, which had more than 75% mortality among Indian major carps, was found mainly due to \textit{A. hydrophila} (Lakshmanan \textit{et al.}, 1989).

In aquaculture, especially \textit{P. aeruginosa} and \textit{P. fluorescens} have been considered as opportunistic pathogenic species (Alderman and Polglase, 1988; Angelini and Seigneur, 1988) however, other species of the genus may also be serious opportunistic pathogens including \textit{P. anguilliseptica} in eel, \textit{Anguilla japonica} (Wakabayashi and Egusa, 1972), \textit{P. chlororaphis} in amago trout, \textit{Oncorhynchus rhodurus} (Hatai \textit{et al.}, 1975) and \textit{P. plecoglossicida} in ayu, \textit{P. altivelis} (Kobayashi \textit{et al.}, 2000). Mohammed (1999) isolated \textit{Pseudomonas aerugenosa} from apparently healthy \textit{Oreochromis niloticus} and diseased fish.

Tripathy \textit{et al.} (2007) isolated \textit{Pseudomonas aeruginosa} from intestine of freshwater fish and from pond sediment. \textit{Pseudomonas fluorescens} is known to be part of the normal flora in the intestines of tilapia. \textit{Pseudomonas putida} caused a disease similar to columnaris disease caused by \textit{F. columnare}, bacterial coldwater disease caused by \textit{F. psychrophilum} and motile \textit{Aeromonas septicemia} (MAS) caused by \textit{Aeromonas sp.} (Plumb, 1999). \textit{Aeromonas hydrophila} causes skin ulcers at any site on the fish and often they are surrounded by a bright red rim of tissue (Plumb, 1999); however, ulcer caused by \textit{P. putida} can be observed almost exclusively on the dorsal surface of the fish.

\textit{Pseudomonas anguilliseptica} is an opportunistic pathogen for a variety of fish species cultured in marine and brackish waters worldwide (Daly, 1999). This microorganism was originally described as the causative agent of the red spot disease of Japanese eel (\textit{Anguillajaponica}) cultured in Japan (Wakabayashi and Egusa, 1972). Since then, the pathogen has been isolated in different countries from a variety of cultured and wild fish species such as European eel (\textit{Anguilla anguilla}), black sea...
bream (*Acanthopagrus schlegeli*), ayu (*Plecoglossus altivelis*), Atlantic salmon (*Salmo salar*), sea trout (*S. trutta*), rainbow trout (*Onchorhynchus mykiss*), whitefish (*Coregonus* sp.), Baltic herring (*Clupea harengus membras*), striped jack (*Pseudocaranx dentex*), and orange-spotted grouper (*Epinephelus coioides*) (Austin and Austin, 1999; Daly, 1999). Since 1990, outbreaks of winter disease, a hemorrhagic septicemia also caused by *P. anguilliseptica*, have been reported in farmed gilthead sea bream (*Sparus aurata*) in several Mediterranean countries including France, Portugal and Spain (Romalde et al., 2001; Domenech et al., 1999). The pathogen has also been isolated from turbot (*Scophthalmus maximus*), another valuable marine-fish species cultured in Europe (Lopez-Romalde et al., 2002). Eels and several other species, including goldfish, have been shown to be experimentally susceptible to *P. anguilliseptica* (Inglis et al., 1993).

*Enterobacter cloacae*, an enteric bacterium that belongs to the family Enterobacteriaceae, has been reported as an opportunistic pathogen in humans (Kanemitsu et al., 2007; Breathnach et al., 2006) and other organisms such as fish (Hansen et al., 1990) and insects (Sanders and Sanders 1997). Khan et al. (1987) isolated *Enterobacter* species from alimentary canal of *Clarias batrachus* and *Heteropneustes fossilis* fishes. Kasing et al. (1999) isolated *Enterobacter aerogenes* from intestine of four freshwater fish species belonging to the family Cyprinidae reared in experimental ponds. Hansen et al. (1990) have isolated *E. agglomerans* from the kidney of infected dolphin fish, *Coryphaena hippurus* L showing that this enteric bacterium is pathogenic to fish. Enteric bacteria are not the normal flora in the intestinal tract of fish and hence their presence in the infected fish may be the result of the association of fish with the polluted waters (Geldreich and Clarke, 1966).
*Escherichia coli* and *E. cloacae* have been shown to have possible involvement in the infection of fish (Troast, 1975).

*Escherichia* species are Gram-negative, rod-shaped, non spore forming bacteria belongs to the Enterobacteriaceae family, widely distributed in nature. Kasing *et al.* (1999) isolated *Escherichia coli* from intestine of four freshwater fish species belonging to the family Cyprinidae reared in experimental ponds. Khan *et al.* (1987) isolated *E. coli* from alimentary canal of *Clarias batrachus* and *Heteropneustes fossilis* fishes. *Lactococcus garvieae* (formerly *Enterococcus seriolicida*), besides being zoonotic, is a significant pathogen of cultured yellowtail (*Seriola quinqueradiata*) in Japan as well as a number of other species including rainbow trout and freshwater prawns, *Macrobrachium rosenbergii* (Chen *et al.*, 2001).

*Edwardsiella* species are Gram-negative, rod-shaped bacteria. *Edwardsiella tarda* is the causative agent of Edwardsiellosis in many commercially important freshwater and marine fishes (Lan *et al.*, 2008). *Edwardsiella ictaluri* causes a septicemia in catfish and is a highly contagious disease with serious effects on the commercial culture of catfish in the southern USA (Noga, 1996). *Yersinia ruckeri* is Gram-negative, non spore-forming, straight rod shaped bacterium, widespread in fresh-water environments (Noga, 1996). *Yersinia ruckeri* is the cause of enteric red mouth (ERM) disease, a condition that has been known since the 1950s. ERM has been a problem mainly for cultured rainbow trout, but all salmonids and some other species of fish are affected. Bacterial kidney disease (BKD) is a chronic systemic infection caused by the Gram-positive bacterium *Renibacterium salmoninarum* (Evenden *et al.*, 1993; Fryer and Sanders 1981), characterized by marked affinity for kidney tissue (*Earp et al.*, 1953; Belding and Merril, 1935).
Among *Flavobacterium* sp, three species are considered as primary pathogens to freshwater hatchery-reared and wild fish populations. *Flavobacterium columnare* causes the columnaris disease, *Flavobacterium branchiophilum* causes the bacterial gill disease and *Flavobacterium psychrophilum* causes the bacterial coldwater disease. *Flavobacterium columnare* was formerly called *Flexibacter columnaris*, but in 1996 it was transferred to the genus *Flavobacterium* (Bernardet *et al.*, 1996). Outbreaks of columnaris disease are rarely spontaneous, but are influenced by a combination of environmental (temperature) and other factors stressful to the host, such as high stocking density, high levels of ammonia and organic load (Wakabayashi, 1991). *Flavobacterium columnare* has been reported from neon tetras (*Paracheirodon innesi*) (Michel *et al.*, 2002).

The first publication describing pathology and isolation of the *Citrobacter freundii* from aquarium fish was from the communication by Sato *et al.* (1982). *Citrobacter freundii* was subsequently isolated from diseased Atlantic salmonids in Spain and the USA (Baya *et al.*, 1990). Kasing *et al.* (1999) isolated *Citrobacter freundii* from intestine of four freshwater fish species belonging to the family Cyprinidae reared in experimental ponds. *C. freundii* causes abnormal inflammatory changes in the intestine of trout and inflammatory and necrotic changes in the internal organs of cyprinids. The illness was discovered by means of artificial infection with a pure culture of *C. freundii*.

*Mycobacterium* species are Gram-positive, long rod-shaped, non spore-forming and acid-fast bacteria. The species of *Mycobacterium* that is pathogenic for fish are *M. marinum* in marine fishes and *M. fortuitum* in fresh water and brackish water fishes (Post, 1987). Three *Mycobacterium* spp. (*M. marinum, M. fortuitum and M. chelone*) are common in ornamental fish and have been reported from ornamentals.
in New Zealand (Diggles et al., 2002). Kasing et al. (1999) isolated Bacillus spp from intestine of four freshwater fish species belonging to the family cyprinidae reared in experimental ponds. Enterococcus species are Gram-positive, non spore-forming cocci. They are responsible for considerable economic losses in cultured yellowtail fish (Kusuda and Salati, 1993), turbot (Toranzo et al., 1995) and tilapia. Enterococcus faecium and E. faecalis were the predominate species isolated from fish in the integrated farms, whereas E. casseliflavus and E. mundtii isolates were most prevalent in traditional fish farms (Petersen and Dalsgaard, 2003). Winter ulcers, induced by the fish pathogenic bacterium Moritella viscosa (previously Vibrio viscosus) (Benediktsdottir et al., 2000) have caused financial losses in farmed salmonids and cod, Gadus morhua (L.), in several countries around the North Atlantic (Thorarinsson and Lystad 2003; Colquhoun et al., 2004). Salmonella spp. appear to be common in aquarium water (Manfrin et al., 2001). Selvin et al. (2005) reported that bacterial disease outbreaks particularly ‘vibriosis’ and ‘black shell disease’ impose a significant constraint on the sustainable production of shrimp. Vibrio alginolyticus and V. harveyi poses a serious disease problem in cultured black tiger shrimp in India (Selvin and Lipton, 2003). Larval mortalities occurring in molluscan hatcheries have often been associated with bacterial contamination and more specifically with vibrios (Lipton et al., 2003). The hatchery production of Pinctada fucata was seriously affected by massive larval mortalities caused by Vibrio sp (Subash, 2009). Subash et al. (2007) also revealed that the total bacterial load in the culture tank water was found to increase in the hatcheries of Pinctada fucata during disease out breaks resulting in heavy larval mortality.

The disease in fishes is dependent upon 3 factors: host susceptibility, pathogen virulence and environmental conditions. The experimental infection can be
achieved by introducing the known number of pathogen through several routes: intramuscular, intraperitoneal and bath challenge. However, successful results were obtained by intramuscular and intraperitoneal routes. Under predisposing factors such as poor water quality, high ammonia as a result of high stocking density and feeding, ectoparasites, inadequate handling and stressful conditions, the microorganism found a portal of entry into the fish host (Moraes and Martins, 2004). After successful entry into the host, the pathogen’s ability to withstand the destructive capability of the host can be achieved by the genetic makeup of the pathogen. The classical example for such involvement of genes in the resistance is documented by Crosa et al. (1980).

### 2.5 Haematological changes of host–pathogen interactions

Haematological parameters changes would be sign of fish physiological responses against environmental stresses e.g. such as heavy metals in water pollution (Vosyliene, 1996) or bacterial infections (Austin and Austin, 1987). Often fish bacterial infectious diseases caused by *Aeromonas*, *Flavobacterium* and *Vibrio*, *Edwardsiella* showed septicemia and hemorrhagic lesions. Thus important internal organs such as kidney, spleen, liver and pancreas that have important functions in fish physiology must be affected acutely by infectious pathogens (Austin and Austin, 1987). Therefore hematological changes would occur subsequently in response to the invading pathogens. Haematological tests have shown useful information in detection and diagnosis of metabolic disturbances and disease in fishes (Aldrin et al., 1982). Haematological studies in animal research and in human diseases are well accepted and considered to be routine procedure in diagnoses (Ranzani-Paiva et al., 2001). The study of the haematological picture is frequently utilized for the detection of physiopathological changes in different stress conditions (Nussey et al., 1995).
Periodical checking of the blood will clearly indicate the health status, such as diet deficiency/starvation and stress condition (Rajan and Lipton, 2001).

Haematologic analysis will enhance fish cultivation by facilitating early detection of situations of stress and or diseases that could affect production performance (Tavares-Dias et al., 2005; Rehulka et al., 2004). A number of haematological indices such as haematocrit (Ht), haemoglobin (Hb), total erythrocyte count (TEC) and so on are used to assess the functional status and oxygen carrying capacity of blood stream (Shah and Altindag, 2004). Other studies have previously drawn attention to the importance of enhancing the knowledge of the clinical haematology and biochemistry of salmonids affected by infectious bacteria (Rehulka, 2002; Waagbo et al., 1988; Barham et al., 1980) and viral haemorrhagic septicaemia (Pollnitz et al., 1994; Hoffmann, 1980). These studies focused on the pathological morphology of the blood cell.

The haematological parameters are an important tool of diagnosis that reveals the state of health of fish (Martins et al., 2004; Rehulka, 2002). For example, decreased red blood cells and hematocrit were found in coho salmon (Oncorhynchus kisutch) infected with V. anguillarum (Harbell et al., 1979); in Asian cichlid fish (Etroplus suratensis) with epizootic ulcerative syndrome (Pathiratne and Rajapakshe, 1998); in rainbow trout (Oncorhynchus mykiss) with ulcerous dermatitis (Rehulka, 1998); in rainbow trout experimentally infected with Aeromonas sobria and A. caviae (Rehulka, 2002); in carp (Cyprinus carpio) experimentally infected with A. hydrophila (Harikrishnan et al., 2003) and in Nile tilapia experimentally infected with Streptococcus iniae (Chen et al., 2004). On the other hand, an increase in the white blood cells was observed by Haney et al. (1992) in chum salmon (Oncorhynchus keta) with erythrocytic necrosis virus.
Many authors have described anaemic states in cases of bacterial infection of salmonids. Waagbo et al. (1988) described signs of severe anaemia combined with a reduction of RBC, Ht and Hb in Atlantic salmon Salmo salar suffering from the ‘Hitra disease’. Cardwell and Smith (1971) found a progressive effect on the Ht and Hb in juvenile chinook salmon with vibriosis. Foda (1973) described a reduction of Hb in a severe Aeromonas infection in Atlantic salmon. Barham et al. (1980) recorded a reduction of Hb and Ht in rainbow trout infected with the Aeromonas and Streptococcus bacteria. A decrease in Ht in a bacterial kidney disease in brook trout is also reported by Hunn (1964). The red blood corpuscles, hematocrit and haemoglobin levels were significantly low in Aeromonas, which causes severe skin lesions in rainbow trout (Rehulka, 2002). However, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were normal. The changes in the basic haematological indices may also be induced by some parasitic protozoa, or certain metazoan parasites.

Hoffmann and Lommel (1984) indicated that rainbow trout affected by proliferative kidney disease (PKD) have distinct anaemia with diminished Ht, Hb and RBC. Haney et al. (1992) observed that in Viral Erythrocytic Necrosis (VEN) disease in Chum salmon (Oncorhynchus keta), the total erythrocyte blood count, hematocrit and hemoglobin were decreased but total white blood cell count was increased. Buckley et al. (1976) reported that prolonged reduction in hemoglobin content was deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants or infectious diseases. Tavares-Dias et al. (2002) reported a significant decrease in RBC, Hb, Ht, and MCHC accompanied by an increase in MCV in Oreochromis niloticus with gill ichthyophthiriasis and saprolegniosis. In carp
experimentally infected with *A. hydrophila*, Harikrishnan *et al.* (2003) have related increased WBC counts. According to this author, decreased RBC counts and hematocrit indicate that erythrocytes are being affected or destroyed with the infection.

### 2.6 Oxygen consumption and ammonia excretion changes of host–pathogen interactions

The fishes are more susceptible to different diseases in the restricted environments. Different predisposing factors such as deteriorating environmental conditions, increasing incidence of contact due to overcrowding and competition play an important role in disturbing the respiratory physiology in the fishes. Not much information is available that show the effects of various types of infections which disturb the respiratory metabolism in fishes whereas several publications are available on the effect of pollutants on structural changes in gills/ or oxygen consumption of fish. Waykar and Lomte (2001) studied the respiratory response of freshwater bivalve, *Parreysia cylindrica* to endosulfan and reported that the rate of oxygen consumption was found to be decreased with increase in exposure period the decrease was maximum in chronic exposure as compared to acute exposure. Physiological changes in growth, oxygen consumption, and heart rate of freshwater crab, *Potamonautes warreni* coincided with high levels of microbial infestation and associated pathological changes in the gills of the crabs (Schuwerack *et al.*, 2001). Reduction in oxygen consumption has been reported in *Channa striatus* exposed to organophosphate pesticide (Natarajan, 1981), *O. mossambicus* due to organochlorine intoxication (Vasanthi and Ramasamy, 1987) and *M. cupanus* following carbamide treatment (Arunachalam and Palanichamy, 1982).
Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress (Magare and Patil, 2000). Secretion of mucus over the gill curtails the diffusion of oxygen which may ultimately reduce the oxygen uptake by the animal. If gills would be destroyed due to xenobiotic chemicals (Grinwis et al., 1998) or the membrane functions are disturbed by a changed permeability (Hartl et al., 2001), oxygen uptake rate would rapidly decreased. On the other hand, the metabolic rate (in relation to respiration) of fish could be increased under chemical stress. Kalavathy et al. (2001) reported that the dimethoate is efficiently absorbed across the gill and diffuse into the bloodstream resulting toxic to fish.

Metabolic activities reflect the general state of animals under a certain physiological or pathological condition. The European eel Anguilla anguilla and the Japanese eel A. japonicus infected with C. columnaris showed hyperplasia of gill epithelium and occasional necrosis of gill filaments in advanced stages of pathogenesis (Funahashi, 1980). Grizzle and Kiryu (1993) reported that catfishes suffering from septicemia due to infection with motile aeromonads had enlarged branchial epithelium with significant gill lesions.

Suitable literature on ammonia excretion changes of host–pathogen interactions is not available. The balance between production and excretion of ammonia may be disturbed by various endogenous and exogenous factors (Lloyd 1992). Several studies have shown a decrease in ammonia excretion with the increase in salinity (Wright et al., 1995). In confined aquatic systems, the accumulation of excreted ammonia can lead to decreased growth, increased vulnerability to disease, and pathologic changes in gill structure, among others effects (Wilkie, 1997).
2.7 **Histopathological changes of host-pathogen interactions**

Histopathology is the microscopic study of tissues affected by disease. The procedures adopted for the preparation of material for such studies are known as histological or histopathological techniques. Studies on the tissue level changes as impact to the bacterial invasion, help to provide valuable information about the route of entry of the pathogen, the fate of tissues invaded by the pathogen and which organs are affected by the pathogen.

Histopathological lesions caused by *P. anguilliseptica* infection have been described in European eel (Ellis *et al*., 1983), salmonid species (Wiklund and Bylund, 1990) and cod (Ferguson *et al*., 2004). Among these species the more intense and widespread lesions were observed in Japanese and European eel. In particular, the diseased Japanese eel had severe necrotic haemorrhagic changes affecting the spleen, kidney, liver, dermis, subcutaneous adipose tissue, vascular walls, bulbus arteriosus and heart. Histopathological studies on ulcerative lesions of *Trichogaster pectoralis* (snake skin gourami) confirmed that diffuse proliferative mycotic granulomatosis is a consistent feature of epizootic ulcerative syndrome (EUS) and the results indicated that interaction between rainfall, deteriorating water quality and presence of pathogens could provide stressful conditions for fish, thereby inducing EUS lesions in susceptible fish populations (Pathiratne and Jayasinghe, 2001).

Yambot and Inglis (1994) described an acute mortality among Nile tilapia in which the most apparent clinical signs included opaqueness in one or both eyes, accompanied by exophthalmia and eventual bursting of the orbit. Others have reported adherence of bacteria to intestine (Horne and Baxendale, 1983) and skin (Kanno *et al*., 1989) followed by invasion of the liver, spleen, muscle, gills and intestine.
2.8 Management of disease

Control of fish disease is currently based almost entirely on chemotherapy and it will entirely retain a role in the management of fish culture systems (Roberts, 1995). Anti-bacterial chemotherapy has been applied in aquaculture for over 50 years (Inglis, 1996). Antibiotics are also used prophylactically in carp culture at times of year when haemorrhagic septicaemia is most likely to occur (Inglis et al., 1994). Antibiotics have been used for treatment and prevention of bacterial diseases and the success of treatment depends on antibiotic susceptibility of etiologic bacteria (Yanong, 2006).

Ghosh et al. (2011) found that the antibiotic sensitivity profile of *Pseudomonas fluorescence* was resistant to amoxycillin, cloxacillin, penicillin-G and ampicillin, thus these antibiotics cannot be used as therapeutic agents for treatment. El-Bouhy and Khalil (2008) mentioned that Gentamycin was one of the effective choices for treatment of staphylococcus infection in some freshwater fish. El-Hady and El-Katib (2009) reported that *Pseudomonas fluorescence* isolates are highly susceptible to oxytetracycline. Erythromycin has been used to treat diseases caused by *Streptococcus* spp and others Gram positive bacteria in the food at the rate of 100 mg/kg of fish per day for 20 days (Post, 1987). Each fish culture facilities require extensive studies to determine the best method of diseases control. Test and slaughter, quarantine and restriction of movement, drug therapy and sanitation and immunization and disease resistance are considered as the methods of controlling bacterial diseases among cultured fish (Post, 1987).

Previous studies indicated that *E. tarda* is susceptible to various antibiotics (Mohanty and Sahoo, 2007). Choresca et al. (2011) found that *E. tarda* was susceptible to ampicillin, gentamicin, chloramphenicol, ciprofloxacin and trimethoprim/sulfamethoxazole and resistant to streptomycin and tetracycline. Heo
and Seo (1997) found that the *E. tarda* was susceptible to Ciprofloxacin in food at a dose of 100 mg/kg body weight or more for 3 days. Ibrahem *et al.* (2010) reported that orbifloxacin can be awaited as effective antibacterial agent for control of edwardsiellosis caused by *E. tarda* and the treatment is much more successful when initiated at the earliest time of infection.

Wolska *et al.* (1999) also reported that 99% of *Pseudomonas aeruginosa* strains were susceptible to ciprofloxacin. Sarker *et al.* (2000) performed drug sensitivity test and found that 50% of the *Aeromonas sobria* isolates were highly sensitive to oxytetracycline, oxolinic acid and chloramphenical and resistant to erythromycin and sulphamethoxazole. Kou *et al.* (1988) and Liao *et al.* (1996) used oxytetracycline in aquaculture as bactericide. Lio-Po and Sanvictores (1987) found positive effect of oxytetracycline in controlling *Pseudomonas* sp. in tilapia fry. According to Shariff *et al.* (1996) oxytetracycline (about 20 ppm) in a dip or bath solution is used against bacterial disease in Malaysia and Singapore. Chowdhury *et al.* (2003) found positive effect of Renamycin (oxytetracycline) against bacterial infection. The study in Vietnam by Crumlish *et al.* (2002) showed that all *E. ictaluri* isolates showed either partial or full resistance to oxytetracycline, sulphamethoxazole + trimethoprim and oxolinic acid while drugs that showed sensitive reaction to all isolates were furazolidone, ciprofloxacin, nitrofurantoin, norfloxacn, gentamycin, enrofloxacin, florfenicol and amoxicillin.

Musa *et al.* (2008) showed average antibiotic resistance in ornamental fish to be 41.85% with 23% intermediary and 34.5% sensitive cases. The report on drug resistance of motile *Aeromonas* sp. of fresh water fish farm by Hatha *et al.* (2005) showed that 100% of the bacteria tested were resistant fish bacterial pathogens to ampicillin, 94.5% were resistant to novobiocin, 52.7% were resistant to amoxicillin.
and 40% were resistant to oxytetracycline. Enrofloxacin and oxytetracycline were widely used for treatment of bacterial infection in aquatic animals as the drug of choice, but Jongjareanjai et al. (2009) showed that enrofloxacin (37.04% susceptibility and 51.85% resistant), and oxytetracycline (11.54% susceptibility and 84.62% resistant) had poor efficacy to eliminate the bacteria. Choresca et al. (2011) suggested that proper aquarium hygiene should be observed and in addition, appropriate regulations of physical conditions in the aquarium are required to avoid stress and occurrence of diseases.