2. REVIEW OF LITERATURE

Stocking of stunted Indian major carp fingerling in grow out ponds to maximize production by use of catch-up or compensatory growth and is becoming popular year after year. Culturing of stunted carp fingerling has many advantages like good survival, better and uniform growth performance in short culture time. But there is lack of scientific evidence on the disease resistance mechanisms of stunted Cirrhinus mrigala fingerling.

2.1 Catch-up or Compensatory growth

Catch-up or compensatory growth refers to the fast growth that follows a period of reduced growth resulting from restricted food availability or some other unfavourable environmental conditions (Jyrki Nikkie et al., 2004). It has long been recognized that animals, even organs have propensity to catch up or compensate, following loss or deprivation (Reid and White, 1977). Application of this naturally occurring biological phenomenon could improve growth and survival rate, lessen the culture period and thereby reduce production cost (Jobling and Koskela, 1996; Chatakondi and Yant, 2001). Compensatory growth has been widely reported in salmonids (Miglavs and Jobling 1989, Quinton and Blake, 1990; Maclean and Metcalfe, 2001), carps (Veerina et al., 1993) catfish, Ictalurus punctatus (Kim and Lovell, 1995), rainbow trout, Oncorhynchus mykiss (Jobling and Koskela, 1996; Jyrki Nikkie et al., 2004), Nile tilapia Oreochromis niloticus (Melard et al., 1998), hybrid sunfish (Hayward et al., 2000) Carassius auratus gibelio and Leiocassis longirostris (Xiaoming et al., 2004).

There are different ways of stunting the fish to obtain rapid growth, among them food restriction and crowding are the most important. When growth inhibiting conditions are replaced by conditions optimal for maximum growth, catch-up growth was exhibited (Leatherland et al., 1993). Veerina et al., (1993) reported that fish farmers in Andhra Pradesh produce stunted carp fingerling by stocking early fingerling at high densities with sub-optimal level of feeding and rearing them for 6-12 months. Puttaswamy and Ramesh (1995) have demonstrated the production of stunted major carp fingerling of 28-30 g size by stocking fry at one lakh/ha and rearing for a period of 6 months, with sub-optimal level of feeding and fertilization. The technique of producing stunted fish by stocking at high densities has shown to exert adverse effects on growth (Leatherland and Cho, 1985;
Jorgensen et al., (1993) and performance of coho salmon (Schreck et al., 1985). Vijayan and Leatherland, (1988) have reported significant decrease in food consumption and feed gain ratio of fish stocked at high densities indicating a reduction in the metabolic efficiency. High stocking density is identified as a stressor by many workers for several species (Wedemeyer, 1976; Klinger et al., 1983; Gatlin et al., 1986; Vijayan et al., 1990).

2.2 Stress in intensive aquaculture

The important stress factors in intensive aquaculture systems are water quality conditions, fish culture procedures and biological interactions between fish. Barton and Iwama (1991) stated that within limits fish could survive physical and chemical challenges by expending energy. However, the debilitating effects of many of these stress factors on their physiological condition increase exponentially (Wedemeyer, 1996). Even though, lethal stress will be rare in well managed aquaculture operations, chronic stress that eventually becomes manifested as adverse effects on health and physiological condition is more common. The pathophysiological effects of stress such as impaired resistance to infection, diseases can be insidious (Wedemeyer, 1996).

2.2.1 Stress and immune response

Stressful situations result in a cascade of events that are transduced centrally and communicated via the nervous and endocrine systems (Schreck, 1981; Barton and Iwama, 1991). A number of stressors are commonly associated with intensive aquaculture like crowding, inadequate nutrition and poor water quality (Klinger et al., 1983; Schwedler et al., 1985; Blazer, 1992). These stressors are known to suppress the immune system of teleost fish (Maule et al., 1989).

The physiological stress response involves the release of catecholamines and cortisol. These hormones are known to play a significant immuno suppressive role (Schreck, 1981; Schreck and Li, 1991). Ellis, (1981) reported that stress cause alterations in the physiological system of fish and can affect its defence mechanisms. The complex interactions of stress factors modulating the defence mechanisms are only beginning to be understood and as yet there is little relevant information in this field concerning fish. Stress associated modulations in the immune system of fish have been demonstrated in
Onchorhynchus mykiss (Angelidis et al., 1987), O. tshawytscha (Maule et al., 1989) common carp (Yin et al., 1995), rohu and catla (Mohan Kumar, 2003).

The immune system of fish is a complex network of molecules, cells and organs to defend against pathogenic microorganisms and non-infections foreign substances. Hence literature on the above aspects have been reviewed in detail.

2.3 Defence mechanisms in fish

Fish are living in an aquatic environment which contains pathogens. Any alteration in the equilibrium between the organisms and the environment can make host susceptible and pathogen virulent leading to outbreak of disease. However, under normal conditions the fish maintains a healthy state by defending itself against these potential invaders with relatively well developed defence mechanisms. The defence mechanisms of fish can be broadly dichotomised into specific and non-specific. These two arms of the immune system are not independent but closely interlinked (Ellis, 1989). The immunity which fish derives against a pathogen is the result of a delicate interaction and cooperation between the two defence mechanisms.

2.3.1 Non-specific defence mechanisms

Non-specific defence mechanisms otherwise referred to as the innate defence system, comprises of humoral and cellular components (Ellis, 1989). These include surface barriers like mucus, skin, gill epithelium, gastro-intestinal tract and mucus membrane. The humoral serum factors are complement, C-reactive protein, lectins, transferin etc. The cellular factors consist of phagocyte cells such as neutrophils and macrophages which engulf and eliminate foreign bodies entering the internal systems of fish. This branch of defence system is successful in preventing a wide array of pathogens from becoming systemic (Ingram, 1980; Fletcher, 1982; Ellis, 1989).

2.3.1.1 Integumental innate defences

The integumental innate defenses of fish are important as the portal of entry of pathogens and they may be considered as a barrier to infection in resistant fish or as a primary site of attachment and replication of pathogens in susceptible fish. There are
different ways in which integument and mucosal surfaces confer innate immune protection like mucus, antibacterial peptides, proteases, lectins, lysozymes, etc.

Mucus forms a protective primary barrier between the fish and the environment. It is continually being secreted by specialized goblet cells present in the epidermal layer (Harris et al., 1973). Proteins and carbohydrates have been found to be the major components of mucus similar to mucin of higher vertebrates (Pickering, 1976). Mucus plays a major role in preventing pathogens from attaching to the epithelium and having an opportunity to invade the fish tissues. The mucus layer of fish species is also known to have immunoglobulins (Fletcher and Grant, 1969; Bradshaw et al., 1971; Wold and Selset, 1977). Fish skin mucus contains lysozyme, which can attack the peptidoglycan layer of bacterial cell walls causing them to lyse (Fletcher and Grant, 1968; Fletcher and White, 1973).

The epidermis in fish may also respond to non-specific irritation by thickening of the cuticle or a hyperplasia of the malpighian cells thereby minimizing the chances of epidermal disruption (Ellis, 1989). Presence of C-reactive proteins and complement have been reported in the mucus of some fish species (Harrel et al., 1975; Ramos and Smith, 1978). If any breach in the mucus barrier results in entry and colonization by the pathogenic microorganisms, there are several protein molecules and cells in body fluids which resist against the infection. If pathogens are successful in crossing the integumental defences there are a number of plasma proteins, which may prevent further spread of infection.

2.3.1.2 Complement

Complement is a system of serum proteins which is central to many defence mechanisms (Ellis, 1989). The complement of teleost fish can be activated directly by lipopolysaccharide present in cell wall of gram -ve bacteria and results in lysis of cell membrane of non-virulent bacteria. This is the so called alternative complement pathway (ACP). However, bacteria that cause disease in fish are resistant to being killed by this mechanism can be killed when the complement is activated by the classical complement pathway (CCP). Teleost fish have both the ACP and CCP of activation directly comparable to those of mammals (Yano, 1996). The ACP activity is very high in fish serum compared
with that of mammals. Yano (1996) suggested that this pathway is very important in the defence mechanisms of fish.

2.3.1.3  **Lysozyme**

In fish, it is found mainly in tissues rich in leucocytes such as the head kidney, skin, alimentary tract and gills (Fletcher and White, 1973; Grinda et al., 1988; Hollaway et al., 1993; Yousif et al., 1994). The role of lysozyme in the host defence mechanisms against infectious diseases has been reported by many workers (Fange et al., 1976; Lindsay, 1986; Lie et al., 1989). In plaice, lysozyme is constantly present in the serum and is secreted by monocytes and neutrophils (Murray and Fletcher, 1976). Yousif et al., (1991) have reported high lysozyme contents in the eggs of Coho salmon. Although, the sources of this is not precisely known, it seems likely that it is released from the kidney and other lysozyme rich tissues of the mother and transported to the developing eggs via the serum (Hjelmeland et al., 1983; Grinde, 1989; Yousif et al., 1991).

2.3.1.4  **C-reactive proteins (CRP)**

C-reactive protein (CRP) is the first protein to appear in the plasma of humans and most animals suffering from tissue damage, infection or inflammation. It is so named because it reacts with and precipitates C-Polysaccharide, a component of pneumococcal cell walls in the presence of calcium ions (Volanakis et al., 1990). CRP has also been reported in fish skin mucus and ova (Winkelhake and Chang, 1982; Yano, 1996).

Channel catfish CRP (100 KDa) has precipitated components of cell wall of saprolegnia species as well as CPS in the presence of Ca\(^{2+}\) (Szalai et al., 1994). C-reactive protein is similar to immunoglobulins in several properties, including precipitation, agglutination and promotion of phagocytosis through complement activation (Pepys, 1981). In rainbow trout, the CRP-CPS (C-Polysaccharide) complex suppressed in vitro growth of V. anguillarum and enhanced the phagocytosis of the bacteria through the activation of the complement system (Nakanishi et al., 1991).

2.3.1.5  **Lectins**

Lectins are a group proteins with different specificities for binding carbohydrates. These lectins can agglutinate a number of fish bacterial pathogens (Yano, 1996). Fish
lectins are similar to other vertebrate lectins (Harrison, 1991) and they have been reported in the ova, mucus and serum of many fish species (Ellis, 1999). Voss et al. (1978) reported chinook salmon egg lectin inhibited the growth of different virulent bacterial species that are pathogenic for chinook salmon and other salmonid fishes. In ayu fish, it is reported that skin mucus lectin shows a high affinity to the lipopolysaccharide (LPS) purified from the V. anguillarum cell wall (Itami et al., 1993). A monopause-binding lectin, isolated from the serum of Atlantic salmon has shown opsonising activity for a virulent strain of A. salmonicida (Arason, 1996).

2.3.1.6 Phagocytosis

Phagocytosis is the process whereby cells internalize, kill and digest invading microorganisms. The phagocytic cells form the second line of defence in fish, are associated with elimination of invading microorganisms (Secombes, 1996). Based on morphology, fish phagocytes are classified into monocytes/macrophages and neutrophil granulocytes. Macrophages and granulocytes are mobile phagocytic cells found in the blood and secondary lymphoid tissues (Secombes, 1996). Both produce oxygen free radicals during respiratory burst on contact with or during phagocytosis of bacteria.

2.3.1.6.1 Neutrophils

Neutrophils contain large amounts of myelo-peroxidase, which in mammals is involved in the production of bacterial hypophthalite ions and presumably the same can occur in fish. In fish, neutrophils can be isolated from blood, kidney and spleen. The number of circulating neutrophils vary considerably due to the greater proportion of lymphocytes and thrombocytes in fish blood. It has been established that fish neutrophils are functionally analogous to neutrophils of higher vertebrates. Teleost neutrophils have granular cytoplasm and the shape of nucleus varies in different species from round centric to lobed (Finn and Nielson, 1971a).

Migration and phagocytosis by neutrophils and macrophages during experimentally induced bacterial inflammation in rainbow trout were reported by Finn and Nielson (1971a). There are several reports on the infiltration of neutrophils to wound site (Finn and Nielson, 1971a; Huizinga et al., 1979; Azad et al., 2001; Mohan Kumar, 2003). They also
reported that chemotaxis stimulus is involved in the migration of neutrophils from blood vessels to the site of injury.

2.3.1.6.2 Monocytes/Macrophages

Monocytes are a population of white cells in the blood and kidney. In the body they form 0.1% of leucocytes population (Ellis, 1976; Fergusson, 1976). The presence of monocytes in appreciable number in kidney apart from blood may strengthen the hypothesis that they originate in the haemopoetic organ. Typically, the macrophages in fish are non-nuclear, non-specific, esterase positive and peroxidase negative. Functionally, they can act as accessory cells for lymphocyte responses, are avidly phagocytic, can secrete oxygen and nitrogen free radicals and kill variety of pathogens (Secombes, 1990). Fish macrophages are also capable of taking up soluble antigens like bovine serum albumin particularly in the pronephros (Ellis, 1977).

Many workers have identified highly phagocytic cells similar to mammalian monocytes, but confusion still prevails over these observations due to great difficulty in identifying them. The macrophage is defined as a mononucleated tissue cell, derived from circulating monocytes which adheres to glass and plastic, is characteristically highly phagocytic and has an undulating membrane (Laskin and Lechevalier, 1972; Spector and Marino, 1975). However, Ellis (1977) concluded that if macrophages as a defensive phagocytic population scattered throughout the tissue are to concentrate their numbers rapidly in a localized area of injury, the presence of a population of circulating precursors would be pre-requisite. These precursor cells in blood would be termed as monocytes.

2.3.1.7 Inflammatory response

Inflammatory response is the basic protective response of the host cell/tissue to injury or pathogenic invasion. The response is mainly to prevent the loss of body fluids, further damage of cells and tissues and decay of cells by sealing off the site of infection from other parts of the body and is common to all vertebrates. It is the starting point which ultimately decides the overall resistance and protection. Even the antibody response and cell mediated immunity are decided by the basic inflammatory response. The main requirements of the inflammatory response are that the basic structural integrity of the tissue concerned is maintained, despite the injury and that functional blood supply is
maintained. If there is disturbances in the integrity of tissue and blood supply system, inflammatory response ceases to function.

Depending on the factors that influence inflammation and the extent of coordination rendered possible during inflammation by the specific and non-specific components, the extent of inflammatory response is divided into acute and chronic inflammatory responses. An acute inflammatory reaction to the tissue damage is usually followed by organization of the acute inflammatory exudates to form granulation tissues, which fills the defect left by the removal of the damaged tissue. This granulation tissue is gradually replaced by fibrous tissue, which ultimately produces a fibrous scar at the site of damage. In most cases, the injurious agent is destroyed or neutralized in the earliest stages of the inflammatory response. Sometimes however, the damaging stimulus persists despite the tissue responses directed at destroying or neutralizing it, and further episodes of tissue destruction may result. In such circumstances the changes of tissue damage, acute inflammation, granulation tissue formation and attempts at fibrous repair may all proceed concurrently instead of sequentially. This is known as chronic inflammation. The chronic inflammation with the development of proliferative lesions leading to fibrosis, known as granuloma is the special feature.

2.3.2  Adaptive immune system

The term adaptive relates to the ability of the system to adapt to the microbial challenge. It is also called acquired or specific immunity. It has unique attributes such as specificity, diversity, memory, specialization, tolerance and homeostasis. This is responsible for initiating and mediating the three aspects of specific immunity viz. humoral immunity; cell mediated immunity (CMI) and memory. Humoral immunity refers to the production of soluble antibodies called as immunoglobulins, while CMI refers to response which are mediated by a variety of cells including lymphocytes and other types especially macrophages, which are recruited by lymphocyte products. The memory constitutes an adaptive change in the lymphoid cell population, elicit a secondary response against the same antigen on subsequent challenge which is characterized by shorter latent period and enhanced magnitude (Ellis, 1989; Arkoosh and Kaattari, 1991).

2.3.2.1  Lymphocytes
The cells involved in bringing about the specific immune response are the lymphocytes found in circulation in lymphoid organs and other tissues (Ellis, 1989). In higher vertebrates, the lymphoid cells can be divided into two distinct populations of B and T lymphocytes. Indication of such heterogeneity in fish lymphocyte population exist as in higher vertebrates, but it is not yet clear whether these populations are exactly homologous to the T and B cells of mammals (Ellis, 1982, 1989; Blaxhall and Hood, 1985). The bone marrow equivalent in fish is still in question. The most recent functional and histological evidence strongly suggests that the anterior kidney may play this role though other organs may also participate to some degree (Kaattari, 1992).

Lymphocytes in fish are present in the circulating blood,ymphs and lymphoid organs. Evidence indicates that the integumental membranes (Skin, gill and gut) of fish regarded as immune reactive tissues, show increased numbers of lymphocytes under pathological or physiological circumstances (Peleteiro and Richards, 1985). The immune system of teleost can therefore be divided into a) systemic involving the lymphoid organs and vascular system and b) integumental involving the skin, gills and gut associated with lymphoid cells.

Immune response in vertebrates is a function of interaction and coordination among the body organs and their cellular components. Function of these organs is to produce specific antibodies and other effective mechanisms leading to destruction and removal of invasive material (Anderson, 1974). In teleosts, lymphoid organs which have been implicated to carry out these or related functions are the thymus, kidney and spleen. Integumentary lymphoid system which was underestimated till recently (Lamers, 1986; Ellis, 1989) has been shown to be very important in various localized immune function (Rombout et al., 1993). The cellular components of lymphoid organs of fish are comprised of small and large lymphocytes, granulocytes, monocytes and macrophages (Lamers, 1986).

2.3.2.2 Lymphoid organs

Fish posses a rich variety of lymphomyeloid tissues which resemble lymphoid tissue and bone marrow of higher vertebrates. Predominantly, myeloid tissues occur in the oesophagus and gonads of the elasmobranches and in the head and shoulder region of holocephalians. In chondrosteans (sturgeons), the main lymphomyeloid tissues are thymus,
spleen, anterior parts of kidney, meningeal myeloid tissue, pericardial tissue and lymphoid masses of intestine especially in the spiral valve (Fange, 1982). The teleosts have thymus, pronephrons and spleen as their lymphoid organs (Lamers, 1986; Ellis, 1988b, 1989).

2.3.2.2.1 Thymus

The thymus of teleost is a paired organ located near the branchial cavity and it is extremely superficial being situated within the epithelium of the branchial cavity, external to the basement membrane and extending dorso-laterally in the gill chamber between the first and fourth gill arch (Ellis, 1982; 1989). The thymus in fish can be regarded as a central/primary lymphoid organ as in mammals where T-lymphocytes differentiate into cells with specific roles in defence system. Though the role of thymus in fish is not fully understood, much evidence exist to suggest that its role is similar to that in mammals.

The pool of virgin lymphocytes are produced in the thymus, which then emigrate to join the peripheral pool of lymphocytes in circulation and in other lymphoid organs and appear to be non-executive in function, i.e. they do not participate in antigen uptake or production of antibodies (Ellis, 1988b). Histology of the thymic structure may differ between species, but the cellular components are basically similar (Chilmonczyk, 1992).

2.3.2.2.2 Kidney

Kidney in teleosts is located below the vertebral column and extends anterior-posteriorly, covered by a thin layer of peritoneal membrane. Ontogenically, the kidney of fishes differentiate in the two phases, the head kidney (pronephros) which develops first followed by opisthonephros, the former losing its excretory function as the latter develops (Ellis, 1982). Both parts of the kidney contain a generalized haemopoietic tissue rich in lymphoid cells and granulocytes (Ellis et al., 1976). The haemopoietic tissue of kidney bears a close resemblance to the bone marrow of higher vertebrates but differs in having an active reticulo-endothelial system (Ellis, 1980) and lymphoid components (Kaattari and Irwin, 1985).

Pigment containing cells viz. melanocytes and melano-macrophages are also seen within the haemopoietic tissue (Ellis, 1989). These cells are highly phagocytic to intraperitoneally injected carbon particles. Lymphoid cells of all developmental stages
(Small and large) are present in the haemopoietic tissue. Lymphocytes circulation takes place through distinct tissue in the kidney. After antigen stimulation in common carp, spherical aggregate of pyroninophilic cells appeared in the haemotopoietic tissue and reached peak just prior to peak serum antibody titre (Secombes et al., 1982a) suggesting its involvement in antibody production similar to that in mammals. However, the exact nature and role of pyroninophilic structure is not yet known, but is thought to develop into melano-macrophage centers as in higher vertebrates (Ellis, 1989).

### 2.3.2.2.3 Spleen

The spleen is the only lymph node-like organ to be found in teleosts situated near the greater curvature of the stomach and flexure of the intestine. The specific capsule is fibrous and devoid of muscle and is covered by serous membrane which is composed mainly of the connective tissue (Ellis, 1989). The spleen of teleosts is believed to be the main organ for uptake, processing storing and maturation of erythrocytes, neutrophils and granulocytes (Anderson, 1974).

### 2.3.2.3 Cell mediated immunity (CMI)

It is a specific immunity dependent upon the presence of T-lymphocytes and is responsible for reactions such as allograft rejection and mixed leucocyte reactions (MLR). Cell mediated immunity (CMI) is a function of T-lymphocytes, which act directly as in specific cytotoxicity reactions (T-killer cells) or indirectly, via the antigen stimulated lymphocytes which recruit and activate macrophages. In vivo manifestations of CMI are delayed type hypersensitivity (DTH), transplantation and tumor immunity, whereas in vitro tests are specific contact cytotoxicity, mixed leucocyte reaction, macrophage activation and antigen induced blastogenesis of lymphocytes (Ellis, 1989).

CMI in fish is less understood. Most studies have dealt with transplantation immunity in the form of skin or scale grafting in teleosts (Rijkers, 1982). The phenomenon of graft rejection in fishes have been studied by Botham, et al., 1980 and Rijkers, 1982. The cellular reaction which occurs at the site of grafting is in essence the same as those in mammals (Rijkers, 1982).

### 2.3.2.4 Humoral immunity
Humoral immunity refers to the production of a specific soluble antibody, immunoglobin (Ig) as a result of interaction between B and T-lymphocytes. After antigen stimulation, there is time lag before the first antibody appears in the circulation (Ellis, 1989). During that time, a complex sequence of events takes place and this includes indication of macrophage involvement in the induction phase (Smith and Braun-Nesje, 1982), aspects of antigen trapping (Ellis, 1980, Secombes et al., 1982b; Lamers and De Hass, 1985), ‘T’ and ‘B’ cell cooperation (Avtalion et al., 1980), production and occurrence of lymphocyte proliferation in the lymphoid organs (Manning et al., 1982), induction of helper (Wishkovsky and Avtalion, 1982) and suppressor (Serero and Avtalion, 1978) and production of interleukins (Caspi and Avtalion, 1984). These suggest mechanisms of antibody production similar to those observed in mammals.

The kinetics of humoral immune reactions in fish have been studied in detail (Sailendri and Muthukkaruppan, 1975; Rijkers, 1982). It is important to realize that following immunization the length of the lag phase, exponential phase and decay phase may be influenced by a number of factors like the ambient temperature (Rijkers, 1982), the type (Ingram and Alexander, 1976) and the nature of the antigen (Busch, 1978), the antigen dose (Ambrosius and Schaker, 1964), route of administration (Harris et al., 1973), age and size of the species (Rijkers and Van Muiswinkel, 1977). For example, injection of an optional dose of sheep red blood cells (SRBC) to common carp (24°C) evoked peak numbers of antibody forming cells in the spleen and kidney after 9-10 days (Rijkers et al., 1980a), but rainbow trout (12º-17ºC) needed 14-15 days for the same response (Chiller et al., 1969). However, Rijkers et al, (1980b) reported successful antibody production to SRBC in common carp between 8ºC and 28ºC, but a clear anamnestic response was impaired at temperatures lower than 18ºC. Upon antigen stimulation, antibody producing cells appear in the kidney and spleen and their numbers increase exponentially. A decrease in their numbers usually follows a sharp peak. Serum antibodies usually appear just before the antibody producing cells reach their peak numbers (10-15 days) and titres increase exponentially to reach a plateau (10-30 days). Depending on the type of antigen and fish species, the decrease in antibody titre may be fast or slow (Ellis, 1989). After the second delivery of the antigen, the lag phase is shorter and the response is accelerated and higher numbers of plasma cells and higher serum antibody are recorded in the blood, the concentration being higher than that
observed after primary exposure (Ellis, 1988a). A real anamestic response is observed only when animals are able to form immunological memory after the first contact with antigen.

The nature of antigen has a prominent effect on the humoral immune response. Rijkers et al. (1980b) have reported in carp, the antibody response to sheep red blood cells (SRBC) administered by injection to be the most effective at low and medium doses although it took longer to develop with low dose. Whereas Lamers et al. (1985b) have reported the antibody response to Aeromonas hydrophila in carp administered by i.m. injection was directly correlated with antigen dose and low dose primary induced weak response. It was reported that the immunogencity of different antigens differs between the species (Stolen et al., 1982). The SRBC has been shown to elicit good plaque forming cells in teleosts (Smith et al., 1967; Chiller et al., 1969) as well as haemogglutinating and humoral antibody (Smith et al., 1967; Sailendri and Muthukkaruppan, 1975).

2.3.2.5 Immune response and memory

Memory has always been considered a hallmark of the specific immune system. The first contact with an antigen usually induces relatively short lived effector cells. However, there are also long lived memory cells among the progeny of the original lymphocytes. These memory cells retain the capacity to be stimulated upon second contact with the same antigen. The magnitude of the secondary response usually depends on the amount of primary antigen (Van Muiswinkel and Wiegertjes, 1997). Rijkers et al. (1980b) reported that a relatively low primary dose is usually optimal for memory in carp. The memory has been demonstrated in fish for both humoral and cell mediated immunity (Rijkers, 1982).

The secondary antibody response in fish reveals response that is reminiscent of the IgM response observed in mammals. In carp, the ratios between secondary and primary antibody responses never reach the high levels observed in mammals (Lamers et al., 1985a). Elegant in vitro studies in rainbow trout showed that the ‘B’ precursor cell frequency in fish immunized with the hapten carrier TNP-KLH increased about 15 fold (Arkoosh and Kaattari, 1991). They also reported that memory in fish is probably due to an expansion of the antigen specific precursor cell pool.
Various pathogen models are being used to evaluate response of the immune system from initial activation to final antibody production. In the present study two different antigen models; Freund’s complete adjuvant (FCA) and Aeromonas hydrophila were used. FCA was used to evaluate the inflammatory response, whereas A. hydrophila was used to delineate the inflammatory response, humoral and protective immunity.

2.4 Freund’s complete adjuvant (FCA)

Adjuvant is defined as a substance used to enhance the immune response. Freund’s adjuvant combining paraffin oil and heat killed tubercle bacilli were among the first immunostimulants used in animals that were mixed with immunogens (Anderson, 1972). FCA is widely used in fish immunology to induce an inflammatory response at the injection site. FCA hold the immunogen in the oil globule after injection, permitting a protracted release of sequestered antigen into the animal tissue. (Stills and Bailey, 1991). In rainbow trout, Salmo gairdneri, FCA tend to result in granulomas and open lesions at the site of injection (Munna and Trust, 1983; Cossarin-Dunier, 1985). The inflammatory response of mrigal (Sobhana et al., 2002a) catla and rohu (Mohan Kumar, 2003) injected with FCA showed typical encapsulatory response around the adjuvant droplets at the lesion area.

2.5 Aeromonas hydrophila

Aeromonas hydrophila is an important ubiquitous bacterium, causing motile aeromonad septicemia (MAS) in fish, a disease condition in tropical and sub-tropical Aquaculture systems. Details on motile aeromonad diseases, Aeromonas as an antigen and vaccination studies have been reviewed here.

2.5.1 Disease by motile aeromonads

Motile aeromonad septiceaemia is a systemic disease caused by members of genus Aeromonas resulting in swelling of the body cavity and haemorrhages. This disease is frequently reported in carps, catfishes, milkfish, mullet ayu, tilapia, murrels and seabass. (Joseph and Carnaham, 1994). Motile aeromonads are gram negative free living mesophiles considered as inhabitants of most water bodies and a consistent part of microflora of both clean and polluted environment of fresh and brackish waters.
The principle factor of initiating infection is believed to be predisposing environmental stress and the disease is known to occur in concert with other pathogens and parasites (Noga, 1986; Miyazaki et al., 2001). Hazen et al, (1978) reported the losses due to the red sore disease in large mouth bass which has been attributed to a combined assault of A. hydrophila and the ciliate, epistylis. In addition, A. hydrophila attacks fish as a secondary invader of injured tissue (Richards and Roberts, 1978; Thune et al., 1993).

This disease has a great significance in tropical aquaculture where the environment generally favours pathogen and the chronic morbidity and growth loss caused have significant economic impact (Plumb, 1984 and 1994).

2.5.2 *Aeromonas hydrophila* as an antigen

Number of factors have been suggested to play a role in virulence and disease causing capability of *A. hydrophila*. The number of outer membrane components such as pili, outer membrane protein (OMP) and lipopolysaccharides (LPS) have been suggested to contribute to the adhesion of *A. hydrophila* to host surface. Adhesion of bacteria to host surface has been postulated to be the first step in initiating an infection (Arp, 1988) and is mediated by specialized complementary molecules. Mittal et al, (1980) linked virulence of *A. hydrophila* strain to cell surface characters.

Studies by Dooley and Trust (1988) on occurrence, visualization, isolation and characterization of tetragonally arranged S-layer of virulent *A. hydrophila* isolates found to be immunodominant among other outer membrane proteins (OMP). The lipopolysaccharides (LPS) and S-layer interaction is important as S-layer occurs in association with LPS morphophytes with a number of O-polysaccharides penetrating into their respective S-layer on cell surface (Dooley et al., 1985). Surface protein array the S-layer, is known to be produced by a number of bacteria and constitute the outermost layer of cell envelop (Dooley et al., 1985). Evidence of direct involvement of S-layer in pathogenesis comes from the studies of Kokka et al, (1992) in which a mixture of S-layer negative *A. hydrophila* cells and purified S-layer protein were injected to mice. The S-layer protein was found to be highly pathogenic. Highly virulent isolates of *A. hydrophila* possessed
O-polysaccharide chains of homogenous length carrying a thermostable serogroup specific epitope 0:11 (Sakazaki, 1987).

A variety of virulence factors work in concert to contribute to the overall virulence of this bacterium. Extracellular products (ECP) including toxins, haemolysins, proteases and acetylcholinesterase appear to contribute to the establishment of A. hydrophila infection in fish (Leung and Stevenson, 1988, Angka et al, 1995). Serum resistance is also implicated in helping the bacterium resisting attack by hostile non-specific immunity such as serum and macrophage killing (Mittal et al., 1980; Janda et al., 1984; Leung and Stevenson, 1988).

2.5.3 Vaccination against A. hydrophila

The first attempt using killed and live A. hydrophila against bacterial haemorrhagic septicemia was made by Schaperclaus (1954). Immunization of trout using heat killed A. hydrophila offered good protection against homologous strain even up to seven months after immunization (Post, 1966). Schaperclaus (1970) reported that vaccination with A. hydrophila strain results in protection against homologous strain, but not against heterologous strain. Injection of eels with live/attenuated A. hydrophila produced higher serum antibody levels, followed by formalin or heat killed pathogen and soluble extracts of the pathogen (Song and Kou, 1981). Thune (1980) used a polyvalent sonicated A. hydrophila for immunizing channel catfish by direct immersion which induced protection against homologous injection challenge, but a poor protection to heterologous challenge. Heat killed vaccines of A. hydrophila strain S-10 with or without adjuvant (FCA) were used to immunize Clarias batrachus, producing protective immunity for a period of 4 months (Kasorchandra and Boonyaraptalin, 1985). Lamers et al., (1985b) studied the humoral immune response and memory formation in carp (Cyprinus carpio) using formalin killed A. hydrophila. Maximum antibody titres were observed at day 20. Height of secondary immune response was positively correlated with the highest of the priming antigen dose and also with the combination of corresponding priming and challenge dose. In another experiment, Lamers and De Hass (1985) reported that heat killed A. hydrophila induced higher antibody levels in Cyprinus carpio than formalin killed bacterium. The antibody titres were rather persistent and measurable over a period of eight months. Ruangapan et al, (1986) reported some degree of protection in Nile Tilapia (Tilapia
nilotica) following I.P. injection of formalin killed A. hydrophila after one week post vaccination. No mortality occurred between 2\textsuperscript{nd} and 5\textsuperscript{th} week against an initial challenge compared to 73-80 percent in controls.

Fingerling of Indian major carps catla, rohu and mrigal were immunized (I.M./I.P) using a haemolysin negative mutant of A. hydrophila. Immunized fish challenged by I.P. injection showed good protection against homologous challenge but showed moderate protection to heterologous challenge in mrigal and rohu (Karunasagar et al., 1991). They reported very high antibody titres in catla followed by mrigal and rohu. The nature of antibodies produced in trout after injection, immersion and oral vaccination of live A. hydrophila cells was studied by Loghothetis and Austin (1994). They observed that the antibodies from skin and muscle extracts, skin and gut mucus, serum and bile reacted to formalinized cells of A. hydrophila and the LPS. The antibodies reacted to lesser extent with exopolysaccharides (EPS), flagella, S-layer protein and haemolytic components of the extracellular products.

Clarias gariepinus was injected intraperitoneally with formalin killed inactivated cells of A. hydrophila. Detectable antibody titre was found on 7\textsuperscript{th} day after vaccination which peaked at 28\textsuperscript{th} day and remained at high level till 35\textsuperscript{th} day (Yin et al., 1996). Anbarsu et al. (1998) immunized Mystus vittatus using formalin and heat killed bacterin, the vaccinated group acquired significant protection over control. Fang et al. (2000) utilized major adhesion factor k, a 43 Kda outer membrane protein of A. hydrophila for immunizing Trichogaster trichopterus. The anti-adhesion serum exhibited very strong ability for bacterial agglutination and could significantly inhibit strains of A. hydrophila from invading epithelioma papillosum cyprini (EPC) cells.