2. REVIEW OF LITERATURE

2.1 Prelude to Review

Fish are in intimate contact with their environment, the water, which contains high concentrations of pathogens, making them more susceptible to a wide range of diseases. In aquaculture systems, diseases are currently making a significant impact on the quality and quantity of fish produced. Disease management is one of the vital components of successful aquaculture. Moreover, the movement of infected fish is contributing to spread of disease. Thus, need for regular screening for the presence of pathogens is very essential. Monitoring the immune response of fish to infection or following vaccination also provides useful information for health management. A combination of methods is usually required for a definitive diagnosis of disease. Immunodiagnostic techniques, which detect either pathogens or host antibodies specific to the pathogen, complement both traditional and molecular methods very well. Rabbit antisera and monoclonal antibodies (MAbs) are generally employed in immunodiagnostic methods.

2.2 Diseases of Indian major carps (IMC)

Diseases are the major obstacles in the success and sustenance of aquaculture practices. The root cause of diseases in aqua farming is either stocking at high densities or stress due to over crowding, excess nutrient and metabolite accumulation, deteriorated water quality conditions. (Ellis, 1995; Ayyappan and Jena, 2001). Diseases caused by fungal, bacterial and parasitic agents encountered in carp culture in India have been recorded (Kumar et al., 1986a, b, c; Karunasagar et al., 1986; Das and Pal, 1987; Das et al., 1989; Nayak et al., 1999). Major bacterial diseases include abdominal dropsy, tail and fin rot, columnaris disease and bacterial gill disease (Gopalakrishnan, 1961; FAO, 1983; Shome et al., 2005). Several parasitic diseases namely, myxosporidiosis,
ichthyopthiriasis, dactylogyrosis, gyrodactylosis and argulosis are reported in catla (FAO, 2007). Non-communicable diseases such as hernia in catla, cholelithiasis, and tail deformities (lordosis), cataract and gas bubble disease in rohu have been noted (FAO, 1983). Sahoo et al. (2000) reported mixed infection of saprolegniasis and myxosporidiosis in IMC. The major diseases encountered in catla farming and their remedial measures are listed in Table 1.

2.3 The Immune System of Fish

The immunology of fish is less well understood than that of mammals or even birds, although considerable progress has been achieved in recent years in areas relevant to the vaccination of fish (Lorenzen, 1993). For protection against infections and maintenance of internal homeostasis, fish have relatively well developed immune mechanisms. Cells equivalent to the immunocytes of mammals have been identified in elasmobranches, chondrosteans, holosteans and teleosts (Good and Papermaster, 1964) and it seems probable that the immunological systems of the higher fish are not fundamentally different from those of birds and mammals.

2.4 The Immune Response of Fish

Immune response to infectious diseases fall into two broad categories: non-specific (innate) and specific (acquired) immunity. The immunity which fish derives against a pathogen is the result of a delicate interaction and co-operation between these two defense mechanisms.

2.4.1 Non-Specific Immune Response

The innate immune system is the only defense weapon of invertebrates and a fundamental defense mechanism of fish. The innate system also plays an instructive role in the acquired immune response and homeostasis and therefore equally important in higher vertebrates. The innate system’s recognition of non-self and danger signals is served
by a limited number of germ line encoded pattern recognition receptors/protein, which recognizes pathogen associated molecular patterns like bacterial and fungal glycoproteins and lipopolysaccharides and intracellular components released through injury or infection. The innate immune system is divided into physical barriers, cellular and humoral components. The epithelium of skin, gills and gut are examples of physical barriers. Humoral parameters include growth inhibitors, various lytic enzymes and components of the complement pathways, agglutinins and precipitins (opsonins, primarily lectins), natural antibodies, cytokines, chemokines and antibacterial peptides (Magnadottir, 2006). Several external and internal factors can influence the activity of innate immune parameters. Temperature changes, handling and crowding stress can have suppressive effects on innate parameters, whereas several food additives and immunostimulants can enhance different innate factors (Ingram, 1980; Ellis, 1989).

2.4.2 Specific Immune Response

The specific immune system which is responsible for initiating and mediating the three aspects of specific immunity: humoral immunity, cell-mediated immunity (CMI) and memory. Humoral immunity refers to the production of soluble antibodies called immunoglobulins, while CMI refers to responses 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 Kattari, 1991). Acquired immunity to reinfection is mediated by lymphocytes and affected mainly through antibodies which can neutralize viruses, facilitate phagocytosis of pathogens through opsonisation and activate complement via the classical pathway (Sakai, 1984).
2.4.2.1 Humoral Immunity

Humoral immunity refers to the production of a specific soluble antibody, immunoglobulin (Ig) as a result of interaction between B and T lymphocytes. After antigen stimulation there is a time lag before antibody appears in the circulation (Ellis, 1989). Mechanisms of antibody production in fish includes indications 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), induction of helper cells (Wishkovsky and Avtalion, 1982), suppressor activities (Serero and Avtalion, 1978) and production of interleukins (Caspi and Avtalian, 1984). These observations suggest that the mechanism for antibody production in fish is similar to those observed in mammals.

2.4.2.2 Cell – Mediated Immunity (CMI)

Specific immune responses that are independent of antibody are collectively termed cell-mediated immunity. This 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. Information on CMI is not extensive, but it is clear that antigenic stimulation of lymphocytes to execute CMI responses can take place (Ellis, 1989).

2.5 Immune System of Teleosts

The immune system of teleost is composed of leukocyte subpopulations including B-cells (Irwin and Kaattari, 1986; Koumans-van Diepen et al., 1994), T-cells (Lin et al., 1992; Miller et al., 1994), granulocytes (Bly et al., 1990; Ainsworth, 1992; Hine, 1992; Slierendrecht et al., 1995), thrombocytes (Esteban et al., 1989; Rombout et al., 1996), macrophages (Meseguer et al., 1991; Weits et al., 1997; Romano et al., 1998) and non-specific cytotoxic cells (Evans et al., 1992; Hogan et al., 1996).
In teleosts, humoral activities include complement lysis of xenogenic erythrocytes (Rijkers et al., 1980), secretion of IgM-like Ig (Miller et al., 1985; Castillo et al., 1993; Killie and Jorgensen, 1995), interleukin-1 (Verburg-Van-Kemenade et al., 1995), macrophage activating factor (Francis and Ellis, 1994; Weits et al., 1997), interleukin-2 (Caspi and Avtalion, 1984) and cytokine-activated antimicrobial responses (Secombes et al., 1996; Yin et al., 1997). Cellular activities that have been shown to take place both in vivo and in vitro include phagocytosis (Nakayasu et al., 1995; Leiro et al., 1996), non-specific killing of xenogenic cells (Evans et al., 1992; Hogan et al., 1996), antigen-enhanced cell proliferation (Marsden et al., 1996), antigen processing and presentation (Vallejo et al., 1992) and T-cell activities (Scapigliati et al., 1999).

2.6 Morphology of Teleosts Immune System

The structure and form of immune system in fishes and mammals differ significantly. Fish lack bone marrow and lymph nodes. Instead the kidney is the major lymphoid organ in addition to the thymus, spleen and mucosa associated lymphoid tissue (Press and Evensen, 1999). C-DNA based in situ hybridization revealed that large numbers of IgM positive cells were widely distributed throughout the spleen, head kidney and thymus confirming that these tissues are major sites of antibody production in fish (Saha et al., 2005).

2.6.1 Thymus

In fish, as in other vertebrates, the thymus is a primary lymphoid organ responsible for the production and maturation of thymocytes (Rowley et al., 1988). Thymocytes are mostly T-like cells. The thymus mostly contains T cells and few populations of B cells. There is heavy migration of thymocytes to spleen and kidney in fish. The vascular supply to the thymus is best developed in the medulla and there exists blood–thymus barrier in fish.
2.6.2 Anterior Kidney

As in all vertebrates, the kidney is located retroperitoneally, exterior to the dorsal wall of the body cavity. The foremost part of the kidney in teleost lacks excretory tissue and is often referred to as the anterior or head kidney. The head kidney is an important haematopoietic organ (Fange, 1986) and has morphological similarities with the bone marrow in higher vertebrates (Meseguer et al., 1995). The head kidney also serves as secondary lymphoid organ – a lymph node analogue, important in the induction and elaboration of immune responses (Kaattari and Irwin, 1985). The head kidney is a major producer of antibody and the melanomacrophage accumulations of the parenchyma are able to retain antigens for a long period of time after administration or vaccination (Brattgjerde and Evensen, 1996) which possibly has a role in immunological memory (Press et al., 1996).

2.6.3 Spleen

Spleen consists of a reticular cell network supporting blood-filled sinusoids that hold diverse cell populations including macrophages, lymphocytes, melanomacrophage accumulations and ellipsoids (Secombes and Manning, 1980). Melanomacrophage centers of the spleen are the major sites of erythrocyte destruction which act as the metabolic dumps (Zapata and Cooper, 1990). The ability of melanomacrophage centers to retain antigens for long period, possibly in the form of immuno-complexes has drawn comparisons with germinal centers in higher vertebrates (Agius, 1980).

2.6.4 Mucosa Associated Lymphoid Tissues (MALT)

The mucosa associated lymphoid tissues of teleost include the gut, skin and gill. Teleost lack organized mucosa associated lymphoid tissues but the gut contains populations of leucocytes, including macrophages, lymphocytes, mast cells, granulocytes and plasma cells (Georgopoulou and Vernier, 1986). These cells and tissues have predominantly localized
role in antigen uptake and processing in fish (Rombout et al., 1993; O’donnell et al., 1994).

2.7 Fish Immunoglobulins (Igs)

Serum proteins with antibody activities are called immunoglobulins. Despite their similarities with respect to physical and chemical properties, Igs are heterogeneous (Nisonoff, 1982) and react specifically with their homologous antigens (Schoparclaus, 1986). Ig is found in most of the tissues of fishes including plasma, lymph, skin, gut mucus and bile. In blood, they comprise about 40 – 50% of the total serum proteins. Among the Osteichthyes a tetrameric molecular structure has been described for the Ig (Acton et al., 1971; Marchalonis, 1977). While, among the Chondricthyes the Ig possesses a pentameric structure (Johnston et al., 1971).

The conspicuous switch from IgM to a low molecular weight antibody class (as IgG in mammals) that occurs during the course of an immune response in vertebrates is not manifested in fishes (Isbell and Pauley, 1983). So the predominant antibody types in fish is high molecular weight Ig often referred to as IgM or IgM like because these molecules have a heavy chain isotype similar to the mammalian µ chain (Wilson and Warr 1992; Van muiswinkel, 1995). The structure and function of IgM is of special interest, being the first Ig to appear and the only Ig class in lower vertebrates. A considerable amount of studies have been carried out on antibody production in teleosts (Ridgway et al., 1966; Clem and Leslier, 1969; Sniesko, 1970).

In addition to the most commonly isolated tetrameric Ig, a number of low molecular weight Igs (generally monomers) have been isolated from various species. Data suggests that these low molecular weight Igs can be dicotomised into either simple monomer (Clem and Mclean, 1975; Warr, 1983; Whittington, 1993) or dimmer (Glynn and Pulsford,
or a structurally distinct low molecular weight Ig (Lobb and Clem, 1981). Presence of halfmeric IgM forms have been demonstrated in other teleost (Bromage et al., 2004). A number of teleosts have the ability to produce heterogenous mixture of IgM polymer by simple variation in the degree of disulphide polymerization of monomer or even halfmer subunit. This heterogeneity in the basic structure referred to as redox forms which has important implication for macromolecular assembly processes and for the generation of teleost Ig functional diversity (Kaattari et al., 1998).

Like many secretary proteins, Igs are glycosylated which is one of the most common post-translational modifications, as it can impact structural stability and biological function. On μ chain region of human and murine IgM, there are five glycosylation sites. Among the oligosaccharides, two are high-mannose and three are complex types (Anderson et al., 1985). As for the heavy chain of the teleost fish Igs, they are also glycosylated. Although the carbohydrate composition in some of the fish Igs have been determined (Frommel et al., 1971), there is little information concerning the carbohydrate structures: the heavy chain of the channel catfish (Ictalurus punctatus) contains oligosaccharides with a terminal sialic acid (Ledford et al., 1993). The carbohydrate moiety of Salmo salar and Gadus morhua IgM was estimated to be approximately 12.5% and 10% of the whole molecule respectively (Magnadottir et al., 1997, 2002).

2.7.1 General Structure of Ig

The predominant structure of Ig isolated from teleost fishes is tetrameric IgM. The molecule was originally deemed IgM because of the equivalent electrophoretic mobility of the heavy chain with μ chain of mammalian IgM (Van Muiswinkel, 1995). It is composed of four monomer sub units, each containing two heavy chains (H) or larger sub unit (~ 72 kDa) and two light chains (L) or smaller sub unit (~ 27 kDa;
Each sub unit consisting of a pair of covalently linked heavy chain – light chain dimers (Partula et al., 1996). The spatial configuration of IgM is believed to be tetrahedral (Pilstrom and Bengten, 1996). Sedimentation coefficient of IgM of bony fishes varies between 13 and 16S (Action et al., 1971) and molecular weight between 600 kDa and 900 kDa (Wilson and Warr, 1992). Fish IgM are apparently of similar sizes and circularly polymerized with the binding-site containing “arms” pointing out in electron microscopic analysis (Shelton and Smith, 1970; Acton et al., 1971; Warr, 1983).

2.7.2 Class and Subclass of Ig

In all land vertebrates there are several Ig classes such as IgM, IgD, IgG, IgA and IgE distinguished by different heavy chains termed as mu (µ), delta (δ), kappa (k), alpha (α) and epsilon (ε) respectively. IgG, IgD and IgE exist as monomers consisting of a single four – polypeptide unit. In humans, there are two sub classes of IgA (IgA1 and IgA2) and four sub classes of IgG (IgG1, IgG2, IgG3 and IgG4). Each class possesses different biological as well as structural properties (Nicholson, 1993). The location and number of interchain disulphide bonds also vary among different classes and subclasses of antibodies. The constant portion of the heavy chains is responsible for the distinct biological characteristics associated with the different classes and subclasses of immunoglobulins.

In fish there are only a few classes of Ig and the main types correspond to the mammalian IgM (Turner and Owen, 1993). The five types of immunoglobulins identified in fish are IgM (Warr, 1995), IgD (Hordvik et al., 1999; Hirono et al., 2003), IgZ (Danilova et al., 2005), IgT (Hansen et al., 2005) and IgH (Savan et al., 2005).

2.7.2.1 Immunoglobulin M (IgM)

In teleost fish, the predominant Ig has been traditionally described as IgM-like (Pilstrom and Bengten, 1996). IgM is the only antibody isotype
found universally in gnathostomes (Bengten et al., 1991; Hordvic et al., 1992; Warr, 1995; Nakao et al., 1998) and is the first Ig to appear in phylogeny, ontogeny and as antibody in an immune response. IgM is typically a pentamer in all basic vertebrate taxa except in the actinopterygian fish where it is a tetramer (Bengten et al., 1991; Hordvic et al., 1992; Warr, 1995; Nakao et al., 1998). Such a tetrameric structure with eight binding sites seems to be less efficient in binding an antigen than a pentameric molecule with its ten binding sites. A tetrahedral IgM would also be efficient in antigen binding, because there are binding sites at all possible directions and support for such a structure is found in electron micrographs, where the arms seem to form a tetrahedron (Shelton and Smith, 1970).

Amino acid sequence analysis of IgM has demonstrated significant homology between IgM heavy chains of diverse species of vertebrates, indicating an evolutionarily well-conserved molecule (Rosenshein et al., 1985; Bengten et al., 1991; Fellah et al, 1992; Andersson and Matsunaga, 1993; Lee et al., 1993). Inspite of this homology, IgM displays considerable structural and biochemical heterogeneity. There is variation in the polymorphic arrangement between different vertebrates.

In mammals, IgM is pentameric and has a flat disc configuration where the participating monomers are both intra and interconnected by complete disulfide bonding. In contrast, the degree of disulfide bonding seems to be variable in a number of examined teleost species. Upon denaturation with sodium dodecyl sulphate (SDS), which disrupts non-covalent bonds, a portion of the tetrameric teleost IgM has been shown to split into mono-, di-, and trimers, depending on the species (Lobb and Clem, 1981; Glynn and Pulsford, 1990; Whittington., 1993). In channel catfish, the halfmeric forms have been observed (Lobb and Clem, 1983). Kaattari et al. (1998) proposed that this diversity in quaternary structure could be paralleled by diversity in function and spatial flexibility. Thus,
variation at the level of quaternary structure could potentially endow the protein with a considerable versatility, which in mammals is achieved by the existence of different immunoglobulin isotypes.

2.7.2.2 Other Immunoglobulin Classes

Until 1997, teleosts were thought to possess only IgM (Savan et al., 2005). However, a truncated form of Ig described from Epinephealus itaira (Clem, 1971) and Australian lungfish, Neoceratodus forsteri (Marchalonis, 1969) which corresponding to the truncated forms of IgY found in ducks, Anas platyrhynchos (Magor et al., 1994b). A large chimeric Ig chain, which shows gene sequence similarity to human and murine IgD heavy chain has been described in channel catfish (Ictalurus punctatus) (Wilson et al., 1997; Bengten et al., 2002), Atlantic salmon (Salmo salar L.; Hordvik et al., 1999), Atlantic cod (Gadus morhua) (Stenvik and Jorgensen, 2000) and Japanese flounder (Paralichthys olivaceus) (Srisapoome et al., 2004). An IgH chain with some sequence homology to δ of IgD (Wilson et al., 1997; Hordvik, 1999; Stenvik and Jorgensen, 2000), teleost specific IgH genes named IgZ and IgT (Sakai and Savan, 2004; Danilova et al., 2005; Hansen et al., 2005) and some unusual pattern of genomic organization (Sakai and Savan, 2004; Danilova et al., 2005; Hansen et al., 2005) have been reported.

2.7.3 Forms of Ig

The teleost Ig exists in secretary (sIgM) and membrane forms (mIgM). Membrane IgM molecules are present in the B cell plasma membrane where they serve as antigen specific receptors and sIgM produced by plasma cells is secreted into blood and other body fluids (Ross et al., 1998; Saha et al., 2005). Membrane bound and secreted forms of Ig seems to differ in molecular weight (MW), which is a variation originating from differences in mRNA splicing patterns (Wilson et al., 1990; Hordvik et al., 1992; Pilstrom and Bengten, 1996).
2.7.4 Distribution of Ig

The occurrence of fish IgM is not limited to the serum alone, it can also be found in secretions such as bile and mucus (Wilson and Warr, 1992). Immunoglobulin of teleost is found in the skin (Lobb, 1987; Cain et al., 2000; Hatten et al., 2001), gut (Joosten et al., 1997; Jones et al., 1999; Abelli et al., 1997), gill mucus (Lumdsen et al., 1993), bile (Lobb and Clem 1981; Rombout et al., 1986; Jenkins et al., 1994) and mucus (Fletcher and Grant, 1969). Ellis (1988) suggested that the immune system of fish could be consisting of a systemic and a mucosal part.

Previously it was suggested that Ig found in the mucus of fish may be locally produced rather than derived from serum (Lobb and Clem 1981; Ourth, 1986) but Rombout et al. (1993) and Zilberg and Klesius (1997) hypothesized that mucus Ig was not the result of serum Ig transduction. The cutaneous mucus Ig of teleosts has been described as similar to the systemically produced homologue in terms of the molecular weight of H and L chain components (Rombout et al., 1993; Lobb and Clem, 1981).

2.7.5 Isotype Heterogeneity of Ig

In several teleost species, both H and L chains have been found as different isotypes. Early attempts to exhibit isotype variation in teleosts suggested that channel catfish possess both H and L chain isotypes (Lobb et al., 1984; Lobb and Olson, 1988). This was determined by production and use of monoclonal antibodies that bound specifically to H and L chain antigenic variants. Furthermore, two loci encoding separate L chain (F and G) have been also sequenced (Lobb and Ghaffari, 2000). Moreover, up to three Ig L chain isotypes (Ghaffari and Lobb, 1993; Partula et al., 1996; Ghaffari and Lobb, 1997; Lobb and Ghaffari, 2000; Haire et al., 2000) and two H chain isotypes have been identified in various species (Hordvik et al., 1997).
2.8 Antigens and Adjuvants for Ig Production

Several antigens have been tried to produce Ig in fish either for the convenience of purification or to get higher titer of Ig. Ig have been purified from normal fish sera (Rombout et al., 1993; Estevez et al., 1995; Adkison et al., 1996; Magnaaadottir et al., 1996) or from immunized fish sera with different strains of bacteria (Isbell and Pauley, 1983; Uchida et al., 2000; Rekha, 2000), different particulate antigens like KLH (Ingram and Alexander, 1979), TNP-LPS (Bourmound et al., 1995), DNP-HAS (Grove et al., 2006), mannen binding protein (Al-Harbi et al., 2000), Protein A (Shin et al., 2006), Sephorose A (Estevez et al., 1993), BSA (Elcombe et al., 1985; Swain et al., 2004; Rathore et al., 2006), FMDV recombinant protein (Srinivas, 2002) and using antibodies like goat IgG (Smith et al., 1992; Pallenzuela et al., 1996; Shin et al., 2006) and mouse IgG (Jang et al., 2004).

Concomitant administration of adjuvants with antigens has long been known to manifest significantly superior antibody responses in fish (Anderson, 1992; 1997; Sakai, 1999). Adjuvants such as Freund’s complete adjuvant, Freund’s incomplete adjuvant, mineral oil, yeast glucans, alum, levamisole, quaternary ammonium compound and saponin have been tested in various species of fish (Raa, 1996).

2.9 Antibody Purification Methods

There are several methods of antibody purification (Table 2) and principles of the common methods are detailed below.

2.9.1 Ammonium Sulphate Precipitation

Ammonium sulphate precipitation is one of the most commonly used methods for separating proteins from solutions. This method is generally used as a first step followed by better purification techniques for isolation, purification and characterization of antibodies. This technique is used in combination with other techniques such as gel filtration
chromatography (Navarro et al., 1993; Van der Heijden et al., 1995; Magnadottir et al., 1996; Uchida et al., 2000) and affinity column chromatography (Rekha, 2000). Due to the presence of a complex group of proteins in fish serum, this method singly can not be employed for the purification of immunoglobulin.

2.9.2 Gel Filtration Chromatography

Gel filtration chromatography separates proteins by size (i.e. molecular weight) consists of a column packed with porous polymeric beads and it is one of the most widely used techniques for protein purification. The resolution in gel filtration is dependent on column length, flow rate of the elution, pore size of the gel and the particle size of the gel bead. Gel filtration of fish antibodies were performed using porous beads among which Sephacryl – S -300 is the most widely used (Sanchez et al., 1989; Pilstrom and Peterson, 1991; Furuta et al., 1995) followed by Sephadox G200 (Isbell and Pauley, 1983), S- HR- 300 (Watts et al., 2001) and Sepharose (Ingram and Alexander, 1979; Coossarini – Dunier 1985; Olsen and Jorgensen, 1986). The technique is widely used as single step purification for fish Igs (Isbell and Pauley 1983; Israelsson et al., 1991; Estevez et al., 1993; Romestand et al., 1995; Bourmaud et al., 1995; Scapigliati et al., 1999; Watts et al., 2001). It can be employed to purify the immunoglobulin even from unimmunised fish sera. Even though this method is effective in purification of fish Ig, the process is time consuming and requires expertise.

2.9.3 Ion Exchange Chromatography

Ion exchange chromatography is both a high resolution and high capacity purification technique. The separation is based on the overall charge of the protein, which is determined by the number of acidic and basic residues in the protein and the pH and ionic strength of the eluting buffer. The parameters affecting resolution in ion exchange chromatography are gel selectivity and particle size, flow rate, gradient...
slope and choice of pH. The columns used for this purpose include DEAE - cellulose (Bradshaw et al., 1971; Oleson and Jorgensen et al., 1986; Sanchez et al., 1989) and monoque anion exchange (Haverstein et al., 1988; Furuta et al., 1995). Researchers have employed this technique for a single step purification of fish Igs (Oleson and Jorgensen, 1986; Haverstein et al., 1988; Sanchez et al., 1989; Furuta et al., 1995; Pettersen et al., 1995; Rajavarthini, 2000).

2.9.4 Affinity Chromatography

Affinity chromatography is by far the most powerful chromatographic technique with respect to resolution and specificity. Affinity techniques exploit the biological properties of the protein. In that the protein of interest specifically binds to an immobilized ligand via some specific biological affinity. It is the only method commonly used to purify antigen specific antibodies from a preparation of polyclonal antibodies (PAbs). The purification is based on a specific antigen-antibody reaction. Several researchers immunized the fishes with antigens and the resultant sera were passed through the columns containing same antigen immobilized on a matrix. The antigen could be a particulate antigens like KLH (Ingram and Alexander, 1979), TNP-LPS (Bourmound et al., 1995), Human serum albumin (Mac Dougel et al., 1995; Grove et al., 2006), mannen binding protein (Al- Harbi et al., 2000), Protein A (Estevez et al., 1993; Shin et al., 2006), antibodies like goat IgG (Smith et al., 1992; Pallenzuela et al., 1996; Shin et al., 2006), mouse IgG (Jang et al., 2004) and BSA (Elcombe et al., 1985; Swain et al., 2004; Rathore et al., 2006). Capture antibodies also can be utilized for effective purification of fish Ig. Zilberg and Klesius (1997) used affinity chromatography to purify Ig from channel cat fish serum using a MAb against the heavy chain of channel catfish Ig. Thuvander et al., (1990) purified rainbow trout Ig using a rabbit anti trout IgM coated affinity column.
2.9.5 Combination of Techniques

Occasionally, single step purification may not be sufficient to purify the Ig from fish serum. In such instances more than one purification techniques should be employed in a stepwise manner. Miyadai et al. (2004) employed extensive dialysis followed by gel filtration for tora fugu slg purification. Shelby et al. (2002) employed polyethylene glycol precipitation and gel filtration for Gulf menhaden Ig purification. Different combinations have been tried including gel filtration; ion exchange and crossed immuno-electrophoresis (Groove et al., 2006), HPLC and affinity column chromatography (Pucci et al., 2003), ion exchange and gel filtration (Action et al., 1971; Sanchez et al., 1993) and ammonium sulphate precipitation, gel filtration chromatography and ion exchange chromatography (Mohanthy et al., 1998).

In addition to the above mentioned methods some unique techniques have been tried to purify fish Igs. Zhong et al. (1999) purified gold fish and carp Ig by polyethylene glycol precipitation. Partula and Charlemagne (1993) used extensive dialysis of the sera for purification of sturgeon Ig.

2.10 Production and Purification of Antibodies in IMC

Swain et al. (2002) purified Ig from non-immunised catla and rohu by ammonium sulphate precipitation followed by centrifugation. IMCs Ig was purified by affinity purification employing ELISA using Aeromonas hydrophila as an antigen followed by gel filtration chromatography (Rekha, 2000). Combination of ammonium sulphate precipitation, gel filtration chromatography and ion exchange chromatography was used to purify rohu Ig raised against Edwardsiella tarda (Mohanthy et al., 1998). Rohu was immunized with a recombinant protein of foot and mouth disease virus (FMDV) and Ig purified by an affinity column coated with the same immunogen (Srinivas, 2002).
2.11 Characterisation of Fish Ig

Characterisation of Ig usually refers to its structure. The success of the structural analysis is critically dependent on the level of purification achieved. The various methods employed for the characterization of Ig is given in Table 2.

2.11.1 Gel Electrophoresis

Gel electrophoresis is one of the most powerful and widely used technique for the characterization of protein.

2.11.1.1 Native PAGE (Poly Acrylamide Gel Electrophoresis)

Native PAGE separates nondenatured proteins which have been employed to find out the purity, integrity and molecular weights of the fish Igs (Adkison et al., 1996; Uchida et al., 2000; Pucci et al., 2003; Rathore et al., 2006).

2.11.1.2 SDS-PAGE

SDS-PAGE (Sodium Dodecylsulfate – Poly Acrylamide Gel Electrophoresis) is a denaturing electrophoresis technique which is used to determine the molecular weights of unknown proteins. Many of the fish Igs were characterized employing SDS-PAGE by resolving the heavy and light chain polypeptides of the Ig (Adkison et al., 1996; Uchida et al., 2000; Watts et al., 2001; Shelby et al., 2002; Cheng et al., 2006; Grove et al., 2006). This technique was also used to demonstrate heterogeneity in heavy and light chain of teleost Ig (Haverstein et al., 1988; Sanchez et al., 1989; Sanchez and Dominguez, 1991; Sanchez et al., 1993; Mac Dougal et al., 1995; Grove et al., 2006).

2.11.2 Chromatography

The molecular weight of both native and reduced proteins can be determined by employing molecular sieving using gel filtration chromatography by comparing the elution peaks with that of standard
reference proteins. Native molecular weight of several fish Igs have been
determined by this technique (Smith et al., 1993; Bourmaund et al.,
1995; Adkison et al., 1996; Palenzuela et al., 1996; Watts et al., 2001;
Rathore et al., 2006). Shelby et al. (2002) employed high performance
liquid chromatography (HPLC) to find out the native molecular weight of
Gulf menhaden.

2.12 Molecular Weight of Fish Ig

Teleostean Ig is primarily a tetramer, composed of four monomer
subunits, each containing two heavy chains (H) or larger subunit (~ 72
kDa) and two light chains (L) or smaller subunit (~ 27 kDa) (Kattari and
Pignelli, 1996). Molecular weight of Ig of some of the teleosts is given in
Table 3. Molecular weight determination of teleost Ig varies considerably
depending on the species and the method employed for estimation.
Magnadottir (1998) reported the molecular weight of Atlantic halibut Ig to
be 840 kDa by gel filtration and 700 kDa by native electrophoresis.
Phillips and Ourth (1986) reported the heavy chain molecular weight of
fish Ig as 72 kDa in contrast to the earlier report of 58 kDa by Hall et al.
(1973).

In teleosts the polymeric Ig was found to be dissociated by
denaturating agents (Kaatari et al., 1998). Upon denaturation with SDS,
a portion of the tetrameric teleost IgM has been shown to split into mono,
di and trimers, depending on the species (Lobb and Clem 1981;
Kobayashi et al., 1982; Glynn et al., 1990; Whittington 1993; Pucci et al.,
2003; Bromage et al., 2004). In some teleosts even the halfmeric forms
have also been reported (Lobb and Clem, 1981; Bromage et al., 2004).
Watts et al. (2001) reported medium molecular weight Ig in tuna. Results
of teleost Ig under various conditions with trypsin and pepsin have
provided important structural information (Van Ginkel et al., 1991;
Magnadottir et al., 1996). Two light chain bands; 27.5 kDa and 28.5 kDa
for sea bass and three bands; 27, 28 and 29 kDa for gilthead sea bream were resolved by SDS-PAGE (Palenzuela et al., 1996).

2.13 Antibody Activity of Ig

Antibody activity of purified Ig was demonstrated by different methods such as ELISA in the case of sea bass and sea bream (Palenzuela et al., 1996), agglutination test in Indian major carps (Rekha, 2000), antigen capture RT-PCR in rohu (Srinivas, 2002) and indirect haemaglutination (IHA) assay in catfish (Swain et al., 2004).

2.14 Stability of Ig

The stability of Ig is affected by a number of factors such as fluctuation in temperature (Acton et al., 1971), enzymatic action (Van Ginkel et al., 1991; Magnadottir et al., 1996) and chemical degradation (Kaattari et al., 1998; Watts et al., 2001). Acton et al. (1971) reported that antibodies were liable to heat at 50°C for 20 min and were dissociated on further storage. Nitzan et al. (2003) reported that upon freezing, the Ig of striped sea bass lost its immunoreactivity in ELISA indicating a drastic reduction in antibody titer. Several reports indicate that a portion of the tetrameric teleost IgM split into mono, di and trimers upon denaturation with SDS depending on the species (Lobb and Clem, 1981; Kobayashi et al., 1982; Glynn et al., 1990; Whittington, 1993; Pucci et al., 2003; Bromage et al., 2004). Hatten et al. (2001) observed that the purified Ig from serum rapidly digested into different small fragments in gut mucus at lower temperature.

2.15 Characterization of Ig of IMC

Earlier studies on L. rohita (Mohanthy et al., 1998; Mohanthy, 2001) and C. mrigala (Sahoo, 2002) revealed the presence of different populations of Ig molecules (IgM type) as tetrameric, dimeric and monomeric forms when subjected to non-reducing SDS-PAGE. SDS-PAGE analysis of IMC Ig revealed molecular weight of 66 kDa and 45 kDa
corresponding to heavy and light chains (Rekha, 2000; Srinivas, 2002). Recently, Swain et al. (2006) has demonstrated a single polypeptide of 210 kDa of rohu IgM from the serum of fry and egg extracts by non-reducing SDS-PAGE which is believed to be the monomer of Ig.

2.16 Serum Ig level in Fish

Measurements of total Ig in fishes can be carried out by simple spectrophotometrical analysis of the purified proteins or by ELISA. Total Ig amounts varied from 2.6 to 16% of the total serum protein (Uchida et al., 2000; Lange et al., 2001). Ig levels in different fishes have been estimated and the results were in the range of 0.25 to 23.5 mg/ml (Israelsson et al., 1991; Magnadottir and Guomundsdottir, 1992; Uchida et al., 2000; Shelby et al., 2002). The total Ig levels vary considerably with individuals, which may be related to size and/or age (Kobayashi et al., 1982; Klesius, 1990), environmental conditions (Olesen and Jorgensen, 1986; Klesius, 1990) or disease status (Magnadottir and Guomundsdottir, 1992; Nielsen et al., 1999).

2.17 Antisera to Fish Ig

Antisera are derived from several plasma cells against different epitopes of the antigen. Antiserum is raised by injecting purified Ig to rabbit, goat or guinea pig in several doses and the blood is collected several days after the first dose and antiserum containing the specific antibody is collected. Antiserum has been used for immunodiagnostic techniques on a large scale (Pomport et al., 1997; Segal et al., 1998; Romestand and Bonami, 2003). Antisera against fish Igs were raised (Table 4) for characterizing antibodies and infection studies. Rabbit or goat is the major sources of such antiserum. Magnadottir et al. (1996) employed a mouse anti Atlantic salmon IgM serum to characterize the Ig. Rekha (2000) produced antisera against IMC Ig in rabbits. Swain et al. (2002) has used the rabbit anti rohu Ig antisera for evaluating the post
vaccination antibody titer against *Edwardsiella tarda* infection in fry and fingerlings of rohu.

2.18 Monoclonal Antibodies (MAbs)

The pioneering work of Kohler and Milstein (1975) on methods for somatic cell hybridization rapidly led to the development of techniques for producing hybridomas secreting specific monoclonal antibodies (MAbs) by fusing normal antibody forming spleen cells (B- lymphocyte) with a mycelia cell using sundae virus (Okada, 1962) or polyethylene glycol (Kao *et al.*, 1974). The basic theory behind the production of MAb is the clonally selection hypothesis according to which each B lymphocyte has the potential to make a monospecific antibody. Now the production of unlimited quantity of specific MAb virtually against any molecule is possible and this technique has a profound impression on all areas of biological research including aquaculture. The main characteristics of MAbs when compared to polyclonal antisera are its specificity, homogeneity and unlimited supply (Campbell, 1984; Goding, 1986). Campbell (1984) has given a comparison of the conventional antiserum and MAb in term of specificity, yield and contaminating antibodies (Table 5).

2.19 Application of MAbs in Fish Health Management

The ability to generate hybridoma cell cultures for *in vitro* production of unlimited quantities of MAbs, homogenous populations of immunoglobulin molecules specific for a single antigenic epitope, has revolutionized diagnostic serology and provided an array of exquisitely specific research probes (Nelson *et al.*, 2000). The MAb in conjugation with ELISA, immunofluorescence and immunoblot is a powerful tool for disease diagnosis. Now MAbs are available for detection of many of the fish pathogens. MAbs can also be used for passive immunization of fish particularly against viral diseases (Lorenzen *et al.*, 1990). MAb are available for determining individual cell surface molecules like fish
thrombocytes, T- helper cells, granulocytes and monocytes (Evens et al., 1988; Bly et al., 1990). MAb technology has helped in the development of potential subunit vaccine for fish against viruses such as IPNV and IHNV (Lawrence et al., 1989; Gilmore et al., 1998). The property of high specificity of MAb has also been utilized for the purification of fish viral proteins (Lawrence et al., 1989; Sanchez et al., 1990).

2. 20 MAbs against Fish Ig

In the past two decades, several studies have been carried out to produce MAbs against several marine and freshwater fish Igs (Romestand et al., 1995). Scapigliati et al. (1999) reviewed the MAbs to Ig and Ig bearing cells. List of such anti fish Ig MAbs and their specificity is given in Table 6. Most of these MAbs recognize the heavy chain of Ig (Lobb and Clem, 1982; Ainsworth et al., 1990; Thuvander et al., 1990; Killie et al., 1991; Israelsson et al., 1991; Pilstrom and Petersson, 1991; Rombout et al., 1993a; Estevez et al., 1994; Koumans-van Diepen et al., 1995; Bang et al., 1995; MacDougal et al., 1995; Pettersen et al., 1995; Romestand et al., 1995; Sanchez et al., 1995; Van der Heijden et al., 1995; Adkison et al., 1996; Magnadottir et al., 1996; Yamaguchi et al., 1996; Scapigliati et al., 1996; Dos santos et al., 1997; Al- Harbi et al., 2000; Morrison et al., 2002; Shelby et al., 2003; Jang et al., 2004; Miyadai et al., 2004; Shin et al., 2006) whereas a few were obtained against the light chain of Ig (Lobb et al., 1984; Sanchez et al., 1993; MacDougal et al., 1995; Romestand et al., 1995; Van der Heijden et al., 1995; Scapigliati et al., 1996; Yamaguchi et al., 1996; Morrison et al., 2002; Shelby et al., 2003). MAbs posses a predominant specificity for the species from which the Ig was obtained (Scapigliati et al., 1996) or cross-react with closely-related species (Thuvander et al., 1990; Adkison et al., 1996). MAbs produced against serum Ig were also found to react with leukocytes (Peterson et al., 1995).
2.21 Applications of MAbs to Fish Igs

MAbs have helped to isolate fish Igs, identify heavy and light chain variants, study the ontogeny of B - lymphocytes and improve techniques for the measurement of fish Ig and specific antibodies (Abs).

2.21.1 Infection Studies

Anti - IgM MAbs have been used to detect specific fish pathogen (Shelby et al., 2002b). The antibody raised against the specific pathogen can be quantified by different immunodiagnostic tools. Experimental infection studies have been carried out to demonstrate the ability of the MAbs to detect specific antibodies (Thuvander et al., 1990; Shelby et al., 2002). Zilberg and Klesius (1997) used channel catfish Ig MAb for the quantification of Ig in the serum and mucus at different age following infection with Edwardsiella ictaluri. Similar works have been carried out in channel catfish (klesius, 1990), masu salmon (Fuda et al., 1991), turbot (Estevez et al., 1995) and rainbow trout (Sanchez et al., 1993) to detect specific pathogens. Specific anti parasitic antibodies were also detected by anti fish Ig MAbs (Leiro et al., 1996; Bobadilla et al., 2004). Bobadilla et al. (2004) employed an anti-turbot IgM MAb for the development of immunohistochemistry and ELISA for detection of circulating antibodies against a myxozoan parasite.

2.21.2 Vaccine Response Studies

Stromsheim et al. (1994) applied MAb based ELISA to monitor humoral response in salmon after bath vaccination against a Vibrio anguillarum. Wagner et al. (2001) employed ELISA to study the antibody response to A. salmonicida vaccination using MAb against salmonid Ig. Post vaccination antibody production has been evaluated against Vibrio anguillarum by different authors employing anti fish Ig MAbs (Coeurdacier et al., 1997; Joosten et al., 2000).
2.21.3 Cross Reactivity and Species Specificity Studies

Cross reactivity of one anti fish Ig MAb with other fish Igs has been reported (Thuvander et al., 1990; Israelson et al., 1991; Mac Dougal et al., 1995; Al - Harbi et al., 2000; Morrison et al., 2002). Adkison et al., (1996) reported that MAb against white sturgeon Ig light chain reacted with other sturgeon light chains but not with any of the teleosts. Cross reactivity of anti Ig light chain MAbs of several fishes suggest that the light chain Ig genes may be highly conserved than those for the heavy chain (Israelsson et al., 1991; Sanchez et al., 1993; Estevez et al., 1994). It has also been demonstrated that there is a significant homology between the amino acid sequences of IgM µ chain from very diverse species (Bengten et al., 1991; Andersson and Matsunaga, 1993; Lee et al., 1993). Another possible explanation of the cross reaction is that the MAbs are directed towards glycosilated region and differential glycosilation pattern accounts for the disparity in binding. In contrast to the cross reactions, Israelsson et al. (1991) reported seven MAbs against the heavy chain of Atlantic cod Ig showing no cross reaction with serum from any other gadiform species.

Cross reactivity between epitope occurs more frequently with serum than with MAbs, demonstrating phylogenetic conservation (Israelsson et al., 1991) or convergent evolution (Marchalonis et al., 1992) of special useful epitopes. The degree of similarity between reactive epitope has been referred to as immunological distance (Israelsson et al., 1991). Cross reactivity of one anti Ig MAb with other fish Ig can be used for the detection of the second fish Ig. Cross reactivity of anti trout Ig MAbs has been used to detect Atlantic salmon Ig in ELISA (O’Dowd et al., 1999; Bricknell et al., 1999).
2.21.4 Studies on Heterogeneity in Heavy and Light Chains of Ig using MAbs

The variations in characteristics of different Igs were demonstrated using MAbs. In white sturgeon the anti light chain MAbs recognized different molecular weight L-chain variants in Western blot (Adkison et al., 1996). Similarly, heterogeneity in teleosts light chain molecular weight has been demonstrated in rainbow trout (Sanchez et al., 1989; Sanchez and Dominguez, 1991; Sanchez et al., 1995), red drum (Mac Dougal et al., 1995), channel catfish (Lobb et al., 1984) and eel (Van der Heijden et al., 1995). Pettersen et al. (1995) depicted the presence of three heavy chain bands in Atlantic salmon using anti Ig MAb. Jang et al. (2004) reported the heterogeneity in both heavy and light chains in flounder Ig. Magnadottir et al. (1996) studied the epitope diversity in Atlantic salmon Ig by MAbs.

2.21.5 Studies on Factors Influencing Antibody Titer

Influence of different factors on the antibody production in fishes was evaluated employing MAbs. Cuesta et al. (2004) studied effect of immunomodulators on total serum IgM level in sea bream employing MAb. Scapigliati et al. (1999) employed a MAb against sea bass Ig to study the Ig levels in relation to age, season and water oxygenation. Pettersen et al. (2005) studied the effect of sea water temperature on leucocytes population in Atlantic salmon post-smolts using MAb against Ig. Pettersen et al. (2003) employed MAb against Ig to study the peripheral blood and head kidney leucocytes population during out of season parr - smolt transformation and seawater transfer of Atlantic salmon. Milston et al. (2003) studied the immunomodulatory effects of environmental factors on the humoral response in Chinook salmon employing anti rainbow trout IgM MAb based flow cytometry. Coeurdacier et al., (1997) employed anti IgM antibodies to evaluate antibody production with time in sea bass immunized with Vibrio
anguillarum. A MAb based sandwich immunoassay was developed to quantify low levels of rainbow trout (Sanchez et al., 1993) and turbot Ig (Estevez et al., 1995) at different age / size. Desvaux et al. (1987) studied the antibody diversity in trouts obtained by gynogenesis or self fertilization by comparing the heavy chain spectrotypes using MAb against heavy chain of trout IgM.

2.21.6 Studies on Lymphoid Organs

Several anti Ig MAbs have been employed to study the origin of production of IgM in fishes. The gut associated lymphoid tissues of carp (Rombout et al., 1993) and sea bass (Abelli et al., 1997) have been demonstrated using MAbs against Ig. MAbs were employed to find out the Ig positive cells in tissues such as head kidney, spleen and blood in different fishes (Secombes et al., 1983b; Koumans-van Diepen et al., 1994; Pettersen et al., 2000; Jang et al., 2004; Bromages and Kaattari, 2006).

2.21.7 Studies on Serum and Mucus Ig

Anti fish IgM MAbs raised against serum Ig were also used to study the difference in serum and mucus Ig. Cain et al. (2000) confirmed the reactivity of mucosal and serum IgM using an anti- trout serum Ig MAb. The results suggest that the early mucosal responses in trout may consist of heterogeneous forms of Ig differing from characteristics of serum Ig. MAbs were also employed to differentiate serum and mucosal Ig (Cain et al., 2000; Hatten et al., 2001; Bromages et al., 2006; Drennan et al., 2007) and their quantification under different infections (Klesius, 1990; Fuda et al., 1991; Sanchez et al., 1993; Estavez et al., 1995; Joosten et al., 1997; Zilberg and Klesius, 1997). Zilberg and Klesius (1997) utilised a MAb to channel catfish Ig for the quantification of Ig in the serum and mucus at different ages following infection with Edwardsiella ictaluri. Drennam et al. (2007) employed a white sturgeon anti Ig MAb for the
characterization of both serum and mucosal Ig against the white sturgeon iridovirus.

### 2.21.8 Ontogeny of Humoral Immune Parameters in Fish

The first appearance of cytoplasmic and surface IgM in lymphocytes varies considerably in different fish species. The availability of specific MAbs has enhanced the knowledge of the ontogeny of Ig-bearing cells in fishes. Employing MAbs several studies have determined the first appearance of the antibodies in egg, embryo, spawn and fry by using different immunological tests such as immunohistochemistry, immunofluorescence, flowcytometry and immuno-electron microscopy (De Luca et al., 1983; Secombes et al., 1983a, b; Razquin et al., 1990; Rombout et al., 1993a; Castillo et al., 1993; Koumans-van Diepen et al., 1994a; Breuil et al., 1997). Conventional ELISA also employed for this purpose (Sanchez and Dominguez, 1991; Castillo et al., 1993; Sanchez et al., 1995).

### 2.21.9 Other Applications of MAbs

MAbs have also been employed to find out surface Ig positive lymphocytes (Lobb and Clem, 1982; Thuvander et al., 1990; Adkison et al., 1996) and their heterogeneity among fishes (De Luca et al., 1993). Magnadottir et al., (1996) demonstrated the reaction of MAb to separate epitopes on salmon IgM heavy chain by partial digestion of the Ig with different enzymes like trypsin, pepsin and papain. MAb has been employed to find out the total Ig level in fishes such as sea bass (Romestand et al., 1995; Scapigliati et al., 1996; Dos santos et al., 1997; Ceurdacier et al., 1997) and eel (Iida et al., 1991; Stromsheim et al., 1994; Estevez et al., 1995). Hayman and Lobb (1993) employed anti cat fish Ig MAbs to demonstrate the distribution and localization of Ig in eggs of cat fish. Estevez et al. (1993) studied the protein A binding characterization of rainbow trout Ig by MAb. Activation of catfish B-cells induced by membrane Ig cross-linking was shown by using an anti-Ig
MAb (Van-Ginkel et al., 1994). Milston et al. (2003) used a fluorescein isothiocyanate labeled anti-rainbow trout IgM MAb to determine the ability of the lymphoblasts to express surface IgM. In addition, MAbs have been used to describe surface immunoglobulin positive cell populations through flow cytometry for resting blood cells in the white sturgeon (Acipenser transmontanus; Adkison, 1996).

2.22 Measurement of Immune Response of Fish

Accurate quantification of the total serum Ig concentration is essential for research in fish diseases, particularly for evaluating antibody production in response to infection and for investigating the possible effect of vaccination on antibody production (Fuda et al., 1991; Sanchez et al., 1993).

2.22.1 Traditional Techniques

Fish Ig have been quantified by traditional immunological techniques like agglutination test, neutralization test, immunodiffusion technique, fluorescence antibody technique and immunoelectrophoresis (Rekha, 2000, Swain et al., 2004). The most widely used traditional quantification procedure is single radial immunodiffusion assay using antiserum (Ingram and Alexander, 1979; Olsen and Jorgensen, 1986; Havarstein et al., 1988; Fuda et al., 1989).

2.22.2 Modern Techniques

2.22.2.1 ELISA

ELISA was developed by Engvall and Perlmann (1971) and is based on the concept of the specific immunological reaction of antibody with antigen. ELISA was introduced in fisheries by Smith (1981) to detect Aeromonas salmonicida and subsequently by Dixon and Hall (1983) to detect IPN virus. Since then many have developed ELISA for detecting different pathogens in fishes. The sensitivity of ELISA was found to be six times higher than the agglutination method for detection of antibodies in
serum of Japanese flounder (Furuta et al., 1995). ELISA is believed to be the best method to measure specific antibody titre in fish (Morrison and Nowak, 2002) among which competitive ELISA is the best with high sensitivity and specificity (Dixon et al., 1994). Rekha (2000) standardised the ELISA for the determination of Ig titre in IMC using PAb.

2.22.2.2 Immunodot

Immunodot technique is similar in principle to ELISA except the use of a nitrocellulose membrane as the solid support instead of microtitre plate. This technique also widely used for the detection of many fish pathogens (Ristow et al., 1991; Shankar et al., 2000; Cesar et al., 2000; Anil et al., 2002). However, reports on the detection of fish Ig using immunodot are very few.

2.22.2.3 Western blot

In ELISA and immunodot false results may occur due to non-specific binding of antigen/antibody to solid phase or background. To overcome this, the target proteins are first separated by SDS-PAGE and then transblotted on to a nitrocellulose paper for specific detection of the separated protein. Western blot is efficiently used for specific determinations of various fish Ig (Thuvander et al., 1990; Sanchez et al., 1993; Romestand et al., 1995; Mac Dougal et al., 1995; Adkison et al., 1996; Scapigliati et al., 1996; Al - Harbi et al., 2000; Morrison et al., 2002).

2.23 Anti- Fish Ig MAb Based Techniques

Antibody productions in fishes have been evaluated by different techniques employing anti Ig MAbs such as ELISA (Thuvander et al., 1990; Iida et al., 1991; Stromsheim et al., 1994; Romestand et al., 1995; Estevez et al., 1995; MacDougal et al., 1995; Van der Hejden et al., 1995; Magnadottir et al., 1996; Leiro et al., 1996; Scapigliati et al., 1996; Dos santos et al., 1997; Coeurdacier et al., 1997; Wagner et al.,
...2001; Shelby et al., 2002; Bobadilla et al., 2004), ELISPOT (Koumans-van Diepen et al., 1995, Joosten et al., 1997), FAST capture ELISA (Zilberg and Klesius, 1997), flow cytometry (Koumans-van Diepen et al., 1994a; Milston et al., 2002), ELISAPLaque (Waterstrat et al., 1991), immunocytochemistry (Lobb and Clem, 1982; Egberts et al., 1983; Secombes et al., 1983b; De Luca et al., 1983; Thuvander et al., 1990; Koumans-van Diepen et al., 1994a,b, 1995; Van der Hejden et al., 1995; Romestand et al., 1995; Scapigliati et al., 1996; Dos santos et al., 1997) and Western blot (Mac Dougal et al., 1995; Adkison et al., 1996; Al-Harbi et al., 2000; Morrison et al., 2002;). But ELISA is the most widely applied test for detection of fish Ig.

2.24 ELISA for Detection of Fish Igs

Detection and quantification of antibodies in fishes can be performed either employing an antiserum (Hamilton et al., 1986; Furuta et al., 1995; Saifi and Hosetti, 1996; Rekha 2000) or MAbs (Klesius et al., 1991; Wagner et al., 2001; Shelby et al., 2002; Bobadilla et al., 2004) against the specific fish Ig employing ELISA. Specific antibodies against a particular antigen can be detected by an indirect ELISA (Klesius et al., 1991; Saifi and Hosetti 1996). Rekha (2000) has standardized ELISA to detect specific Ig in IMCs using antiserum. Rabbit anti rohu Ig antisera was used by Swain et al., (2002) evaluating the post vaccination antibody titre in rohu against Edwardsiella tarda infection in fry and fingerlings of rohu.

2.25 MAb Based ELISA for Detection of Fish Igs

ELISA has been developed to quantify Ig against different antigens (Thuvander et al., 1990; Estevez et al., 1995; Wagner et al., 2001; Shelby et al., 2002b; Bobadilla et al., 2004). Van der Hejden et al. (1995) demonstrated the suitability of the anti European eel Ig MAbs to detect antigen specific antibodies using ELISA. Zilberg et al. (1997) demonstrated the antigen specificity of catfish Igs employing an anti cat
fish IgM MAb based FAST ELISA. Scapigliati et al., (1996) demonstrated the specificity and cross reactivity of MAbs against sea bass immunoglobulins employing ELISA titration curves. Sandwich immunoassays were developed to quantify low levels of rainbow trout (Sanchez et al., 1993) and turbot Ig (Estevez et al., 1995) employing anti Ig MAbs.