General Haematology

Dhar (1948) carried out first work on haematology of an Indian fish, *Channa punctatus* and also conducted preliminary studies on the morphology of corpuscle erythrocyte, leucocyte counts and clotting time. Since then different workers have carried out haematological works on various fishes.

The morphology of erythrocyte in an air breathing fish, *Saccobranchus fossilis* was studied by Banerjee (1956, 1957). Das (1958) studied the haematological parameters of three Indian major carps, *Labeo calbasu, Catla catla* and *Cirrhinus mrigala*. Preston (1960) studied the red cell values in the Plaice, *Pleuronectes platessa* L. and found that haemoglobin and packed cell volume vary both with season as well as size of the animals.

Pradhan (1961) reported haemoglobin and haematocrit values of some important fishes like *Tilapia, Channa, and Heteropneustes*. Banerjee (1966) studied certain haematological parameters of both sexes of *Anabas testudineus*. Qayyum and Naseem (1967, 1968) made observations on routine haematological parameters of carps and Khan and Qayyum (1969) studied the differential blood cell counts in four species of air
breathing fish. Blaxhall (1972) reviewed selected literature regarding their use of haematological techniques in fresh water fish pathology.

Blaxhall and Daisley (1973) gave detailed account of some haematological methods employed for examining blood of *Salmo trutta* L. The parameter they studied included haemoglobin estimation, haematocrit, erythrocyte counts, erythrocyte sedimentation rate and total and differential leucocyte counts. Blood cell morphology of erythrocyte and leucocyte of *Colisa fasciatus* and *Garra gotyla* was studied by Pandey (1974 a, b). Marked intraspecific variations were observed by Joshi and Tandon (1977) in various blood parameters viz. total red and white blood cells, haemoglobin concentration of fresh water fishes under similar ecophysiological conditions.

Van-Varun and Halting (1978) studied the haemoglobin contents of carp *Cyprinus carpio*, yellow fish *Barbus lolubi* and two species of mud fish *Labeo umbratus* and *Labeo capansio*. Mahajan and Dheer (1979 a, b) made a year long study based on monthly observations of haematology of female *Channa punctatus* with respect to haemoglobin, haematocrit values, total erythrocyte and leucocyte count. They also made different enumeration of various leucocytes viz. small and large lymphocytes, monocytes, neutrophils, eosinophils and basophils.

Sex related variations in total erythrocyte count, total leucocyte count, haemoglobin level and packed cell volume in 12 Indian fresh water fish species were studied by Joshi (1980). He noted that except for total leucocyte count all the parameters were higher in males than females. Joshi (1981) studied total erythrocyte count (TEC), total leucocyte count (TLC) and Hb content in a teleost, *Wallago attu* and found these parameters to be related with the size of fish.

Effects of repeated weekly blood sampling on blood parameters were studied by Hoffmann and Lommel (1984) in salmonid *Salmo gairdneri* and cyprinid fish *Leuciseus idus* over a period of 22 days. They observed increased red cell destruction in case of *Leuciseus idus* compared to *Salmo gairdneri*. Choudhary *et al.* (1986) investigated the size and sex related variations of some blood parameters of an exotic fish, *Sarotherodon mossambicus*. While working on differential red blood cell counts, Joshi (1986) found abundance of erythroblasts in normal circulating blood of some fresh water fishes. Rambhaskar and Rao (1987) reported that active fishes generally exhibit higher values of
erythrocyte numbers, haematocrit and haemoglobin. Al- Hassan et al. (1990) proposed peak values of haemoglobin concentration and haematocrit in *Silurus triostegus* which were found to be 9.42 g/100 ml and 25.23% respectively for the males and 9.40 g/100 ml and 25.20 % respectively for females.

Electron microscopic studies on the morphology of blood cells of a freshwater eel, *Amphipnous cuchia* was made by Ahmed et al. (1990). Leamaster et al. (1990) made an exhaustive study on haematology and biochemistry of *Sarotherodon melanotheron*. On the basis of their studies, they reported that number of white blood cells depend on the quality of aquatic environment.

Joshi and Sharma (1991) observed that in hill stream fish *Noemachilus rupicola*, blood values viz. TEC, TLC, Hb, PCV and MCHC which rose during the summer months had an inverse relationship with MCH and MCV which have depicted a fall in their values during that very period. Ranzani-Paiva (1995) while studying the differential leucocyte count (DLC) of the mullet *Mugil platanus* reported lymphocyte to be the most frequent cells in the peripheral blood of the fish. Collazos et al. (1996) studied the seasonal changes in haematological parameters in the blood of *Tinca tinca*. They reported significant changes in RBC and haematocrit in males compared to females during different seasons. Ohja and Pandey (1997) evaluated ultra structural morphology of blood leucocytes in *Clarias batrachus*.

Age related changes in haematology of hybrid striped bass were studied by Hrubec et al. (2001). They stated that while the values of PCV and RBC indices were significantly lower in younger fish, total WBC and lymphocyte count remained significantly higher in fish at 6 and 9 months of age compared to that of neutrophils and monocytes which observed higher values at 6, 9 and 15 months of age. Ueda et al. (2001) noticed seven types of cells viz. erythrocytes, thrombocytes, neutrophils, eosinophils, basophils, lymphocytes and monocytes in the blood of *Oreochromis niloticus*.

Haematological and biochemical analyses of blood in carp *Cyprinus carpio* L. was performed by Svetina et al. (2002). They reported that haematocrit and haemoglobin values of carp fry significantly increased from June to September during the first year of examination. Orun et al. (2003) compared variations in haematological parameters of fish
Alburnoides bipunctatus F., Chalcalburnus mossulensis and Cyprinus macrostomas and reported differences to be species specific as well as gender based.

Ranzani-Paiva et al. (2003) observed the differential leucocyte count in ‘dourado’ Salminus maxillosus. The mean percentages of leucocytes were determined according to sex and stage of gonadal maturation (immature, in maturation, mature, spent and resting). Analysis of leucocyte profile during gonadal development was found to show significant differences between male and female specially the granulocytic cell. The relationship between haematocrit and some biological parameters of the Indian shad, Tenualosa ilisha was determined by Jawad et al. (2004). They found haematocrit values to show a quadratic relationship to fish size (body length), increment up to length of 400mm, after which, it was found to decrease.

Daily variations in haematology and blood biochemistry in healthy male and female tench (Tinca tinca) during four seasons of year were studied by De Pedro et al. (2005). They observed that RBC, Hct and Hb exhibited a similar daily profile in spring and summer season. Moreover total and differential leucocyte counts show significant daily fluctuations depending on the season. Kori-Siakpere et al. (2005) studied haematological characteristics of the African snake head Parachanna obscura. Gao et al. (2007) compared diploid, triploid and tetraploid specimens of loach Misgurnus anguillicaudatus with reference to haematological variations. He observed no significant difference in haematocrit among polyploids but observed a decrease in erythrocyte count with an increase in ploidy level.

Gabriel et al. (2007) assessed the haematological values of Sarotherodon melanotheron from the brakish water creek of Buguma Niger Delta. They gave an account of values of Hb (6.44±0.43dL⁻¹), Hct (20.80±0.43%), Leucocrit (Lt) (6.93±0.29%), WBC (29.64±0.67×10⁹ cells L⁻¹), RBC (2.53±0.03×10¹²cellsL⁻¹), MCHC (31.36±0.9gdL⁻¹), MCH (25.60±0.81Pg), MCV (81.66±1.81fl), thrombocytes (173.93±3.46%), neutrophils (35.8±0.85%), lymphocytes (46.09±1.01%) and monocytes (2.25±0.09%).

Aras et al. (2008) observed monthly variations in haematology and serum biochemical parameters of Leucisus cephalus and reported that TEC, TLC, Hb, Hct, MCV, MCH and MCHC exhibit peak during the month of May (summer season) on the
basis of their studies. They also concluded that these parameters were affected by many endogenous as well as exogenous factors such as reproductive cycle, metabolic rate and water temperature.

Haematological analysis of common carp (*Cyprinus carpio*), old fish (*Carassius auratus*), Tilapia (*Oreochromis mossambicus*) and stinging cat fish (*Heteropneustes fossilis*) was carried out by Dhanaraj *et al.* (2008) following injection of *Aeromonas hydrophila*. They observed that WBC showed a linear decrease from 0 to 21<sup>st</sup> day whereas RBC exhibited a linear decrease from 0 to 2<sup>nd</sup> day in *C. carpio*, *C. auratus* and *H. fossilis* but in *O. massambicus* it decreased from 0-7<sup>th</sup> day and increased from 7<sup>th</sup> day to touch high level on 14<sup>th</sup> day simply to decrease again up to 21<sup>st</sup> day.

Ikechukwu and Obinnaya (2010) studied the blood parameters of African lungfish, *Protopterus annectens* during various reproductive stages (fingerlings, juveniles, intermediates and adults). Intraspecific haematological relationships in *P. annectens* indicated a high positive correlation between haematocrit and erythrocyte count and Hb contents respectively in all size classes of *P. annectens* with mean r-values of 0.060 and 0.43 (P<0.05) for Hct/EC and Hct/Hb<sup>4</sup> respectively.

Singla *et al.* (2012) studied the monthly variations in total erythrocyte counts in some freshwater fishes in Pilli reservoir. Peripheral haematological parameters such as erythrocytes, leucocytes, thrombocytes (TEC, TLC, TTC), haemoglobin content (Hb), haematocrit (Hct), erythrocyte sedimentation rate (ESR), RBC/WBC ratio, MCV, MCH and MCHC of *Puntius filamentosus* in relation to sex, maturity stages, standard length of the fish and season were evaluated by Vijaykumari and Murli (2012). They observed that peripheral haematological make up was not much affected by sex of the fish but however most of the parameters (TEC, TLC, Hb, Hct) registered higher mean values in males than in females. Karimi *et al.* (2013) studied the effect of sexuality on some haematological parameters of the yellowfin seabream, *Acanthopagrus latus* in perician Gulf.

**General Immunology**

Using Jerne Plaque technique in rainbow trout Chiller *et al.* (1969) identified small circulating cells which were useful in antibody production. They further reported that antibodies against fish pathogen are produced by specific cells which either reside in
the anterior kidney, spleen or in small clusters in other areas of the fish or circulate throughout the body of fish via the humoral system.

The pacific hag fish *Eptatretus stoutii* was reported to exhibit a variety of immunologic responses such as allograft rejection and serum antibody production against soluble and cellular antigens by Riviere *et al.* (1975). They also reported the presence of phagocytic and antigen receptive cell production associated with pharyngeal velar muscles in the thymus of young fish, *Eptatretus stoutii*.

O’Neil (1981) observed the primary and secondary humoral immune response to a single intra peritoneal inoculation of MS2 bacteriophage in teleost *Notothenia rossii* maintained at 2°C in experimental aquaria. He observed that all the specimens examined were immunocompetent and produced IgM like neutralization antibody.

Tartner and Manning (1982) using electron microscope studied the thymus gland in trout *Salmo gairdneri* and showed that the membrane separating the thymus gland from external water current was not specialized but merely a continuation of the pharyngeal epithelium.

Ageing changes in lymphopoietic and myelopoietic organs of the annual cyprinodont fish, *Nothobranchius guentheri* and *Cynolebias adloffi* were studied by Cooper *et al.* (1983). The histopathological observations revealed increased amounts of connective tissue, epithelial cysts and myoid cells but decreased number of thrombocytes. They also advocated that as fish ages there is atrophy of the thymus, the immune system gets less vigorous and there is an increase in the incidence of cancer.

Histological and ultra structural studies of the thymus of the dog fish, *Scyliorhinus canicula* were carried out by Pulsford *et al.* (1984). Studies revealed the presence of paired lobular masses above the first two gill arches of embryonic and newly hatched dog fish, involuting at approximately 3 weeks. They also observed that thymus did not have any clearly defined demarcation between cortex and medullary regions. Ultra structural investigations showed the tissue to be composed mainly of a range of different sized lymphocytes with in a connective tissue capsule and reticular epithelial cell framework.

Nakanishi (1986) studied the seasonal modulation in antibody production in relation to the state of lymphoid tissue development in the marine teleost *Sebastiscus*
marmoratus. He further reported antibody levels in fish immunized in summer to be higher than those in fish immunized in winter, even if environmental temperature was kept constant. They also stated that antibody titre is inversely related to the weight of thymus, with the exception of females during the spawning period.

Paired thymic organs of post larval and adult specimens of the Antarctic silver fish Pleuragramma antarcticum and sea bass Dicentrarchus labrax were examined by O'Neil (1989). Histological studies revealed that in both of fish species studied paired thymic anlagens were infiltrated by small lymphocytes at the earliest post larval stages being relatively more advanced in D. labrax. Studies further revealed that at adult stage, D. labrax did not show an increased involution of thymic zones but in P. antarcticum, the organ regressed leaving a few aggregations of small lymphocytes in the outer thymic zone.

Bodammer et al. (1990) examined the spleen and head kidney of striped bass Morona saxatilis and observed that spleen was composed of loosely organized reticulum that supports the red and white pulp. The principal haemopoietic and mature blood cells observed were erythrocytes, erythroblasts, lymphocytes, macrophages, thrombocytes and aggregates of pigmented macrophages. In addition, limited numbers of neutrophils, neutrophilic myelocytes (immature lymphocytes), plasma cells, eosinophils and monocytes were also identified. Reticular cells and fibres appeared to provide a structural framework for both cells and blood sinuses in the spleen. Like spleen, the head kidney, too, contained erythrocytes, macrophages, monocytes, lymphocytes and other less frequently observed cell types.

Holladay and Smith (1996) reyorted that in several species of wild and laboratory exposed Nile tilapia, there is alteration in monocyte chemotactic ability, chemiluminescent response and phagocytosis, which they stated find association with exposure to polycyclic aromatic hydrocarbons and organometals. They also stressed that parameters of immunity may serve as potential sensitive markers of chemical exposure in fish.

A study was carried out by Cubero and Molinero (1997) to elucidate the mechanisms underlying in immunological response of fish after handling, confinement and anaesthetic exposure. Handling caused changes in the immunological cell populations
of the thymus, spleen and pronephros. Stronger response in immunological cells was obtained when handling was followed by confinement. The anaesthetic exposure caused less immunological response thereby reflecting its protective role.

Morphology of the immune system in teleost fishes was studied by Press and Evensen (1999). They observed that the kidneys, thymus, spleen and mucosa associated lymphoid tissue including skin and gills are major lymphoid tissues in teleosts.

Romano et al. (1999) studied the structure of the developing thymus of a marine teleost Diplodus puntazzo by light and transmission electron microscope. They reported that thymus shows sign of development by day 20 post fertilization (pf) as a group of undifferentiated cells. Thymus, however, observed significant increase in size around day 51-66 (pf) and differentiation of cortex and medulla occurred concomitantly.

A technique involving hypotonic lysis of erythrocytes for the purification of leucocytes from the anterior kidney of rainbow trout (Oncorhynchus mykiss) was used by Crippen et al. (2001). For pronephros, 1:5 (w:v) dilution and lysis in hypotonic solution for 10-40s was effective in eliminating erythrocytes. Total leucocyte yield from the pronephros by the use of hypotonic lysis was comparable with that obtained from typical density gradient centrifugation and cell viability was 97% or greater.

Lam et al. (2002) described the morphogenic transformation of the developing Zebra fish thymus from 1 week post fertilization (wpf) to 15 wpf and stated that overall morphology of thymus changed from a small pouch like shape at 1 wpf to a conical shape between 2 and 3 wpf before acquiring a more complex shape from 4 wpf onwards. They also stated that there is expansion of thrombocyte population beginning from 1wpf through 2 to 3 wpf as indicated by the apparent increased appearance of different sizes of lymphocytes.

Breeding related seasonal changes in immunocompetence, health state and condition of the cyprinid fish, Rutilus rutilus L. were studied by Kortet et al. (2003). They observed seasonal pattern in immunological, haematological and body condition parameters for male and female fish and also reported that in two populations the relative size of the spleen and chemotaxic migration activity of head kidney granulocytes decreased immediately before and after spawning respectively. This, they added was
accompanied by an increase in haematocrit values and a decrease in the relative body weight in one population due to physiological stress caused due to hyper active state during spawning period. Liu et al. (2004) studied the development of the lymphoid organs of flounder, Paralichthys olivaceus from hatching to 13 months of age. They observed that except for thymus, all organs grew as the fish grew and by two months of age they attained their maximum relative weight. They further observed that organ weight showed a closer correlation to body weight rather than to age as is the case of thymus.

Lymphomyleoid organs of three common Antarctic fish species Trematomus bernacchii, Trematomus nicolai and Chionodraco hamatus were analyzed by Romano et al. (2004). They studied the histological and cytological organization of the main lymphoid organs viz. thymus, head kidney and spleen and compared with the available data for temperate / warm water fish species. Shao et al. (2004) studied the invitro effect of Aeromonas hydrophila on lymphocytes of Carassius auratus and stated that pathogenic bacteria induce apoptosis in fish immune cells. Results indicated the possibility of existence of a novel anti host strategy which might be employed by bacteria based on the elimination of host immune cell through apoptosis.

The kinetics of the temperature or season mediated immunomodulation in the innate immune parameters were found to remarkably fluctuate among the individuals of the fish, Clarias batrachus and at different time periods and temperatures by Kumari et al. (2006). Although a clear seasonal variation was well marked in the innate immune parameters of this species but the fluctuations of all the parameters were not consistent to any of the temperature except for lysozyme levels that remained significantly lower during summer compared to other seasons.

Xie et al. (2006) examined the thymus of the mandarin fish, Siniperca chuatsi with the help of a light and transmission electron microscope. They observed that by 7 dph thymus was packed with lymphocytes and from 12 dph onwards with mucous cells. They further stated that from 23 dph cortical and medullary zones could be differentiated in thymus as also the presence of thymic epithelial cells, lymphocytes with limiting granulocytes in adult fish.

Histological changes in kidney were used to evaluate the health of the Neotropical fish species Prochilodus lineatus by Camarago and Martinez (2007). The studies showed
enlargement of the glomerulus, reduction of Bowman’s space, occlusion of the tubular lumen, cloudy swelling and hyaline droplets degeneration in the kidney. Mohammed et al. (2007) studied the anatomy, histology and ultra structure of the thymus of a dipnoian fish, *Neoceratodus forsteri* using light and transmission electron microscope. They reported that thymic tissue showed clear demarcation into a cortex and medulla with ample vascularization and also reported that major cellular components were epithelial cells and lymphoid cells.

According to Saurabh and Sahoo (2008) lysozyme is an important defense molecule of fish’s innate immune system and acts as first line of defense against a broad spectrum of pathogens. It is an important index of innate immunity of fish and possesses lytic activity against bacteria. They also stressed that lysozyme activity varies with sex, age and size of fish, season, water temperature, pH, infections etc.

Mohamad and Abasali (2010) studied the effect of plant extract supplement diets on immunity and resistance to *Aeromonas hydrophila* in common carp, *Cyprinus carpio*. Five plant extracts (*Inula helenium*, *Tussilago farfara*, *Brassica nigra*, *Echinacea purpurea* and *Chelrodontium majus*) were mixed thoroughly with artificial feeds and fed to healthy common carp for 60 days and then challenged with *Aeromonas hydrophila*. To evaluate the immune responses and résistance against *A. hydrophila* infection of fish, haematological, biochemical and immunological parameters of fish were investigated at 20, 40 and 60 days of feeding and also 10th day post challenge. Results indicated that respiratory burst activity, serum bacterial activity, lysozyme, serum protein, albumin, globulin, WBC, RBCs and Hb content were enhanced in fish fed with herbal diets as compared to control.

Estaban (2012) summarized the current knowledge of the immunological defense present in skin mucosa and reported that skin mucosa acts as an important anatomical and physiological barrier of fish immune system and also defend the body against external hazards.

**Reproductive Biology**

Ovaries act as an indicator of spawning period in the fishes. Accounts of the ovarian development of fishes have been reviewed by many workers (Kisselevitch, 1923;
Mayenne, 1927; Hickling, 1930 and Raitt, 1933). All of them concluded that the measurements of the diameters of eggs in ovaries of those oocytes which have well advanced towards spawning, may give the evidence of duration of spawning in fish whose spawning habits are unknown.

Low temperature has been observed to accelerate the earlier phase of maturity but prevent the sexual maturation and spawning in *Perca fluviatalis* by Morosova (1957). Pathani (1960) studied the fecundity in *Tor putitora* (Ham.) by both gravimetric and volumetric count methods. They stated that the fecundity of *Tor putitora* ranged from 7,076 to 18,525 in fish of length range from 33.90 to 51.70cm. The workers also observed that the fecundity has straight line relationship with total length, body weight, ovarian weight and volume separately.

Tandon (1961) reported that the eggs increase in number with the increase in the size of fish *Selaroides leptolepis*. Sunder raj and Suttukus (1962) noted increase in egg number in *Cnoscin nebulosus* with the advancement in age. Qasim and Qayyum (1963) found *Ophiocephalus punctatus* to lay 25,000 to 40,000 eggs.

Malhotra (1965, 1966) and Jyoti (1972) observed a winter diapause (dormancy) in the ovaries either in immature stage (stage 1) or in complete mature stage (stage III). This diapause they stated gets broken down only when the temperature starts rising during March/ April. Jee (1973) investigated the reproduction pattern and maturation of the gonads of *Betta pugnase* (Cantor) and found that the species mature at 4cm (standard length) or when 110-120 days old. Further, he concluded from his studies that the fish breeds throughout the year but predominantly during the rainy seasons and this seasonal predominance of breeding was more influenced by rainfall than the supply of food.

Morawska (1984) studied effects of temperature fluctuations over prespawning and spawning and stated that it has a definite bearing on the number and fecundity of the successive batches and on cumulative fecundity during the season in *Tinca tinca*. In African catfish, *Clarias lazera*, a temperature of 25°C has been recommended by Richter and Hurk (1982) to be optimum for conditioning of the brood fish.

Carmie *et al.* (1985) concluded that in *Thymallus thymallus* the sensivity to low temperature reduced the rate of final oocyte maturation. They considered temperature to
be the most important exteroceptive factor controlling sexual cycling in temperate fishes of Kashmir valley.

Guraya (1986) reviewed the available information on oogenesis and concluded that Balbiani’s vitelline body in oocyte of different fish species is simply accumulation of different yolk nucleolus, mitochondria, golgi complex, annulate lamellae, multi vesicular bodies and lipid inclusions. Of these inclusions yolk nucleolus constitutes RNA and protein. He further added that it persists as an organized structure and acts as the centre of all metabolic activities before the deposition of yolk. Greely et al. (1988) characterized the regressed ovaries by numerous nets of oogonia displaying prominent mitotic figures as the fish, Fundulus grandis initiate recrudescence in the ovaries. Following them immediately they added, the proliferation of oogonia starts with the rise in diameter. Previtellogenic oocytes accelerate their process and vitellogenesis begins quickly resulting in to a mature oocyte. Spawning was observed by them to be prolonged and extended from the March to August.

Dobriyal and Singh (1989) noted that total fecundity range from 1,710 to 8,050 in Glyptothorax pectinopterus. In year 1993, they recorded the absolute fecundity in Glyptothorax madrasptanum to be in the range of 1,640 to 6,830.

Warm aquatic habitat has been recognized as a requirement for gonadal maturation and ripening in air breathing teleosts, Channa punctatus and Heteropneustes fossilis by Chatterjee et al. (1990). Ntiba and Jaccarini (1990) in Signaus suter reported that spawning takes place one or two months after the beginning of monsoon season. The histological analysis of ovaries revealed that the oocyte development is highly synchronous and their release takes place in shot intervals of time.

Treasurer (1990) distinguished the developing oocytes of Pike, Esox luciasi from resting oocytes by size. He stated that primary germ cells and resting oocytes formed distinct clusters. He further stated that spent ovaries were disorganized with contracted follicles, folded thick tunica walls with occasional presence of residual oocytes. Fully developed and particularly spent ovaries were considered by him to represent synchronous mode of oocyte development.
Velasco *et al.* (1990) observed during their studies on reproductive biology of *Rutilus lemmingii* that the fish spawned in April and May. Following this they added that in June and July the gonado somatic index (GSI) of females again reaches its minimum value. GSI of males though paralleled that of females but due to narrower range of male variably in GSI values as well as the pattern could not be distinctly observed as in females. They also studied the mean individual fecundity in each age class of the population, which was found to be maximum in 4^+^ and 5^+^.

Casadevall *et al.* (1993) observed in *Ohidion barbatum* that the mechanism of ovulation involved separation of follicular epithelium from the membrane of the ovum by contraction of muscular fibres of the external theca. They also stated that the development of the ovaries corresponds to a synchronous kind i.e. during spawning, the ovaries contain simultaneously oocytes of different stages which are expelled as they attain maturity.

Da and Deniel (1993) reported that the red mullet *Mullus rurmuletus* breeds during the late spring up to early autumn, during the annual cycle. They also stated that the previtellogenic phase lasts for 6 months and the secondary phase of vitellogenesis for 3 months and that with the commencement of spawning the process of vitellogenesis in oocytes ceases. Only 20% of the oocytes were observed to be atretic by then and only a single batch of oocyte was seen to enter the secondary vitellogenic phase. They further added that the spawning takes place in batches, but there was no recruitment of previtellogenic oocytes to vitellogenic phase during this period.

Hoque and Hossain (1993) estimated the fecundity of *Mystus vittatus* from the Rajshai city. They found it to vary from 2,534 to 60,746 with mean fecundity to be 8,635±790.17. Biesiot *et al.* (1994) in *Rachycantron canadum* stated that the previtellogenic stage of oocytes could be subdivided into three stages. These oocytes were characterized by small oocytes with swollen nucleus having nucleoli and the presence of yolk vesicles on the periphery of oocytes.

Batch fecundity (the number of hydrated oocytes) was studied by Casavola (1995), emitted by the fish, *Engroulis encrasiolus* L. in a single spawning act first time in the water of lower Adriatic sea using the hydrated egg method. Agarwal (1996) studied the reproductive aspects of the snow trout, *Schizothorax plagiostomus* and observed cortical alveoli and two types of yolk bodies i.e. lipid yolk and protid yolk. The cortical
alveoli, according to him, originate in the cortical ooplasm and form a conspicuous zone. He also suggested that the cortical alveoli play an important role to check polyspermy in fishes. The most important role of follicular atresia during the normal course of reproduction, he opined, is to limit the number of eggs. Karlou-Riga and Economidis (1997) estimated relative batch fecundity to be 205 oocytes/gm weight by the hydrated and migratory nucleus method.

Bjornsson et al. (1998) reported photoperiod to be one of the controlling factor responsible for the sexual maturation in Atlantic halibut, *Hippoglossus hippoglossus*. Raina (1999), while studying the histology of ovary of *Channa gachua* and *Puntius conchonius*, reported that both of the fish species are partial spawners. She further reported that *Channa gachua* as monsoon spawner and *Puntius conchonius* to be summer/early monsoon spawner.

Dadzie et al. (2000), Ahirrao (2002) and Hussain et al. (2003) were of opinion that the gonadosomatic index can be used as an index for studying the spawning season in the fishes, which changes with the specific gonadal cycle. Sarker et al. (2002) observed significantly highest and lowest mean fecundity to be 21,589 (19,394-23,481) and 11,887 (11,436-12,712) in fish of length range 20 to 22cm and 10 to 12cm in *Mystus gulio*. Thapliyal (2002) while working on *Pseudechenesis suleatus* noted the fecundity to be 1,299-5,885.

Gupta and Gandotra (2003) observed the combined effect of the temperature and photoperiod on induction of ovarian maturation in juvenile *Channa punctatus*. The data generalised clearly showed that the oocyte maturation could be advanced when exposed to warm temperature and long photoperiod. Temperature, they added not only play a significant role in the oocyte maturation but in semen quality as well.

While studying the reproductive cycle of *Osteobrama cotio cotio*, Hussain et al. (2003) proposed that maximum GSI value is attained in the month of June (15.310) followed by a sharp decline in the month of September (3.79) which indicated maximum gonadal growth and spawning respectively.

Bobko and Berkeley (2004) during studies on the reproductive biology of *Sebastes melanops* estimated the absolute fecundity and relative fecundity (based on
fertilized eggs) to range from 2,99,302 embryos for a 6 year old female to 9,48,152 embryos for a 16 years old female. The relative fecundity (based on fertilized eggs) was found by them to increase with age from 374 eggs/ gm for fish age 16.

Victor et al. (2004) studied the fecundity and sex ratio of fresh water fish, *Puntius filamentous* (Valenciennes) collected from Tamparapani River, Tamil Nadu. They found the fecundity of this fish to range from 527 (TL, 87 mm and weight 8.26 gm to 2,925 (TL, 119mm and weight 8.3gm) while mean fecundity to be found by them was 1,054. They also stated that the number of ova increased with an increase in the length of the fish. Further they inferred that the fish was medium fecund and the number of eggs produced was more or less directly proportional to the total length, total body weight, ovarian length and weight.

Behera et al. (2005) carried out studies on the fecundity of stripped Gourami, *Collisa fasciatus* (Bloch and Schneider). Based on their studies they concluded that the weight of ovary is the index of fecundity in comparison to total length, body weight and length of ovaries of the fish because they recorded highest correlation between fecundity and ovary weight.

Roy et al. (2005) in their studies on reproductive periodicity of *Mystus cavasius* (Ham.) found that active breeding season (April- May) are characterized by the higher mean gonadosomatic index (GSI), higher mean gonadal length index (GLI) and increased diameter of the ova. They observed maximum percentages of gravid females (36.46% and 30.13%) in the month of May and June on the basis of which inferred that March to August appears to be the breeding season of *Mystus cavasius* with a peak in May 15th to June 15th.

Research conducted on the maturity of the *Lutjanus fluviflammus* by Shimose and Tachihara (2005) revealed that GSI of most fish were high from April to July (main spawning season), peaking in May and June for both sexes.

Kiran and Puttaiah (2006) noticed higher gonado somatic index values in the month of October, April and June. Ova diameter studies indicated more than one modal group of developing ova in the advanced stage of maturity. Males were reported by them to mature at 108-113mm and females at 88mm.
Fecundity and sex ratio of *Botia dayi*, Hora from Garhwal hills was studied by Kumar *et al.* (2006). They observed striking relationships between fecundity and body parameters. The fecundity of the fish was observed in a range of minimum 2,225 to 8,840 for the fish measuring 10.1cm and 11.5cm and weighing 11.72 and 38.6gm respectively.

Suresh *et al.* (2006) studied the biology of *Macrognathus pancalus* (Ham.) and found that 50% of the males matured at length ranging from 19-11 cm and females at 11-12cm with fecundity ranging from 227 (fish 10.7cm and 5.03g) to 8310 (fish 17cm and 30.31g), while the relative fecundity was 45-274.

Phillips *et al.* (2007) observed the total fecundity in *Fundulus diaphanous* to increase with both size and age. The mean total number of eggs produced were observed to be 526±37 at age 1 (67 mm± 1.8), 744± 3 at age 2 (79 mm± 2.4) and 1062± 43 at age 3 (93mm ±4.1).

Rath *et al.* (2007) during their studies on gonadal development in *Labeo rohita* (Ham.) found that GSI dropped to 2.45 on the next day of spontaneous breeding, which subsequently increased to 14.1 by 45th day of spontaneous spawning. They concluded on the basis of their studies that GSI can indicate maturity status as well as breeding period of *Labeo rohita*.

Shendge and Mane (2007) studied the gonado somatic index (GSI) and spawning season of fresh water fish, *Puntius sophore* (Kher) and found GSI to range between 0.22 to 5.49 in females and between 0.05 to 2.05 in males. The GSI reaches its peak in both male and female during the spawning period, commencing from August and October.

Gandotra *et al.* (2008) reported that in *Barilius vagra* (Ham.) fecundity is more related to ovarian weight(r = 0.971) than other body parameters viz. fish length (r = 0.965) and fish weight (r = 0.908). Reasoning it out they held that is influenced mainly by the ova present in them.

Shendge *et al.* (2008) carried out studies on the gonads of *Clarias batrachus* (Linn.) and reported a direct relationship between the GSI and spawning season but an inverse relationship during post spawning season. They observed the maximum GSI values in the higher length group of fishes i.e. 190-229mm and minimum GSI in lower length group i.e. 150-159mm.
Gandotra et al. (2009) studied the fecundity of *Schizothorax richardsonii* from Rajouri district (J&K) in relation to fish length, fish weight, ovary length, ovary weight and ova diameter. The higher values of correlation coefficient in all the studied parameters indicated a strong relationship between them. However, the relationship between fecundity and fish length was found by them to be highest than all other parameters.

Changes in the ovarian conditions of *Dentex hypselosomus* were examined by Yoda and Yoneda (2009). They observed that the species spawned more or less throughout the year, with its peak during spring to autumn. Further the comparisons of the developing oocytes and degenerating post ovulatory follicles in the ovaries suggested that most females spawned repeatedly over 2 to 3 consecutive days during the peak of the spawning season and concluded that the fish is multiple spawner.

Ghafari and Jamili (2010) studied the reproductive biology of *Barbus pectoralis* and observed from the gonado somatic index that the breeding time in fish extended from January to February with the absolute fecundity ranging from 7,144 to 3,32,196.

Vohra (2011) studied the reproductive cycle of *Esomus danricus* and *Rasbora rasbora* and Subba and Meheta (2012) conducted a study to study the reproductive biology of *Xenentodon cancila*. Plasma sex steroid hormonal profile and gonadal histology were correlated to the annual reproductive cycle of catfish *Hemibagrus nemurus* by Adebiyi et al. (2013).

**Stressors (natural and anthropogenic) and haematology**

Houston and Dewilde (1968) studied the effect of temperature on the haematology of carp, *Cyprinus carpio* during winter, summer and autumn season. They observed a direct relation between temperature and haematological parameters viz. RBCs, PCV and Hb. Whereas MCV exhibited an increase at very low temperature. They added MCH and MCHC volume remained almost stable at moderate to high temperature. Khan and Siddiqui (1970) studied the effect of asphyxiation on the blood constituents of *Channa punctatus* at 15 minutes interval till 75 minutes. By that time they added fishes actually became dead. Mckim et al. (1970) observed an increase in erythrocyte count and haemoglobin content on exposure of fish rainbow trout to copper.
Weinberg et al. (1972) studied the influence of starvation on the peripheral blood cells and blood cell morphology of red paradise fish, *Macropodus opercularis*. Donaldson and Dye (1975) reported an increase in cortisol level on exposure to heavy metal. They observed a decrease in WBC count in general and of lymphocytes in particular.

Agarwal and Srivastava (1976a, b, 1978) studied the effect of cold shock stress on the blood cells of *Colisa fasciatus*. They observed that cold shock for one minute resulted in leucocytosis. They however, observed normal leucocyte count at 3, 17, 12, 19, 315 and 363 minutes and at 12 and 24 hours.

Choubey et al. (1976) documented the effect of light and dark period of haematological profile of *Heteropneustes fossilis*. Effect of heavy metal on the erythrocyte count and haemoglobin concentration of certain teleost was studied by Qayyum et al. (1976). Panigrahi (1977) reported a significant decrease in RBC count, Hb and Hct values in *Anabas testudineus* on exposure to mercury.

Srivastava et al. (1979) described the effect of chromium on the blood parameters of fresh water teleost, *Colisa fasciatus*. Acute effects of copper on the blood of a teleost were studied by Mishra and Srivastava (1980). Blood dyscrasia attributable to copper poisoning were observed in *Colisa fasciatus*, a freshwater teleost after 96 hrs exposure to 3mg/l of copper nitrate and 96 hrs exposure to 4mg/l of the same metal. TLC, Hct, Hb and thrombocytes increased significantly while there was a significant decrease in number of lymphocytes.

Raizada and Singh (1981) studied the effect of starvation on the blood of *Ophiocephalus punctatus*. Mahajan and Dheer (1983) studied changes in haematological values based on weekly samples from a group of starved fish *Channa punctatus* (Bloch.). They observed an increase in RBC and its related values and in total leucocyte population as an initial response to food deprivation. From 5th week onwards however, a sharp decline in these cell population was observed by them.

Marked changes in the haematology of *Anabas testudineus* when exposed to three sublethal concentrations of titanium has been studied by Nair et al. (1984). Sopinska (1984) studied the effect of three stress producing factors viz. capture, transportation and starvation on haematological parameters of carp. Stress of capture was observed by them.
to result in an increased MCV and Hct. They reported an increase in leucocyte count especially the neutrophils and basophils. Further while transportation was observed to result in an increased erythrocyte count, Hct, Hb, Leucocyte count and neutrophil percentage whereas long lasting deprivation of food resulted in decrease of all these haematological parameters.

Gill and Pant (1985) reported decrease in RBC count, Hb percentage and PCV values in *Puntius conchonius* after exposure to mercury. Joshi (1985) studied the effect of dessication (by keeping the fish out of water) on the haematological parameters of fish, *Heteropneustes fossilis*. He noticed elevation in TEC, Hb and TLC count after dessication for 60 minutes.

Dheer et al. (1986) noticed the macrocytic hyperchromic anaemia and leucopenia in fish *Channa punctatus* as an outcome of NaCl stress. Flos et al. (1987) observed an increase in haematocrit values in different fish species after an exposure to zinc.

Thakur and Pandey (1987) studied the effect of 10% sea water (pH 8.8) on fifteen blood constituents of *Channa punctatus* and noticed significant increase in values of erythrocytes and packed cell volume of MCV, MCH and MCHC. Blood glucose and total cholesterol contents were however reported by them to decrease abruptly. Garg et al. (1989) reported an increase in leucocyte count in *Heteropneustes fossilis* after exposure to manganese.

Jewet et al. (1991) investigated the effect of hyperoxia in rainbow trout *Onchorhynchus mykiss* cultured under hatchery conditions. Ahmed et al. (1992) reported decrement in circulating erythrocytes in *Oreochromis niloticus* on exposure to ammonia. Wilson and Taylor (1993) noted phenomenon of haemoconcentration in the blood of rainbow trout on exposure to acutely lethal copper exposure. A decrease in RBC count, Hct and Hb content was observed by Shakoorei et al. (1994) in *Ctenopharyngodon idella* on exposure to Hg$_2$Cl$_2$. They observed an increase in MCV after four weeks of exposure. Singh (1995) reported an increase in leucocyte count on exposure to copper and cadmium in fish *Channa punctatus*. While studying the effect of zinc and lead on the haematological indices of carp, Vosyliene (1999) observed a synergetic effect of these metals on the erythrocyte count, Hb concentration and leucocyte count. Das and
Mukherjee (2000) evaluated the effect of two sublethal concentrations of quinalophos on the blood parameters of fish *Labeo rohita*.

Effect of diazinon on the blood parameters of common carp *Cyprinus carpio* was studied by Svoboda *et al.* (2001). They observed significantly lower values of RBCs, Hb and Hct in exposed groups compared to control groups. They reported a significant decrease in absolute as well as relative leucocyte count in all experimental groups. Carneiro *et al.* (2002) determined the effect of transportation stress on the cortisol, glucose, Hct and white cell differentiation count. The relative percentage of lymphocytes decreased after transportation.

Atamanalp and Erzurum (2003) studied the effect of Manacozeb on the haematological parameters of rainbow trout *Onchorhynchus mykiss* and observed increase in red blood cell numbers and decrease in haemoglobin, MCH, MCHC, PCV, MCV and WBC values. Effect of low temperature and fasting was studied by Sala-Ranabal *et al.* (2003) to evaluate the stress on haematological parameters and plasma composition of gill head sea bream, *Sparas auratus*.

Urbinati *et al.* (2004) evaluated the physiological response of matrinxa (*Brycon cephalus*) after the procedures of capturing, loading and four hour transport at different densities. They observed that though Hct increased after loading but there was no difference in RBC count.

Adult specimens of traira, *Hoplias malabaricus* were subjected to long term starvation and re-fed for 30 days after 90 and 240 days by Rios *et al.* (2005). After 240 days of starvation significant reduction in RBC count, HCT, Hb and red blood cell indices MCV, MCH and MCHC was observed by them. They also observed leucopenia and thrombopenia. After refeeding, the number of leucocytes and thrombocytes recovered but red blood number remained low and there was a significant increase in abnormal cell nuclei.

Effect of sublethal concentrations of zinc was studied by Tyagi and Srivastava (2005) for a period of 135 days on haematological parameters of teleost *Channa punctatus*. The RBC count, Hb, and Hct content was observed by them to decrease but
WBC count, MCV, MCH and MCHC increased during the experimental period. They also stated that alterations in haematological parameters were dose dependent.

Temperature and copper toxicity was reported by Carvalho and Fernandes (2006) to affect haematological response in the Neotropical fish *Prochilodus scrofa* at different pH. They observed that at 20°C, regardless of the water pH, Hct increased while the RBC and Hb concentration decreased compared to control maintained at pH 7.0 at 30°C. The change in the blood parameters, they added, depends on the water pH. Effect of nuvan (dichlorovos), an organophosphate pesticide on the haematological parameters of banded peal spot, *Etroplus suratensis* was studied by Sobhana *et al.* (2006). Blood samples analyzed for different haematological parameters viz. TEC, TLC, Hb, PCV and ESR at fortnightly intervals were observed to record significant reduction (P<0.05) in Hb content in the test groups but differences were not statistically significant. TLC however was significantly higher (P<0.05) in treated groups.

Changes in blood cells of *Clarias gariepinus* after 96 hours exposure to various concentrations of lead nitrate (0, 25, 100 and 200mg/l) were studied by Adeyemo (2007). He observed that PCV decreased significantly while the platelet count increased in treated fishes. Further he noticed a decline in RBC count and an increase in MCV, MCH and MCHC. Acute haematological effects of sublethal levels of Paraquat on the African catfish, *Clarias gariepinus* were studied by Ovie *et al.* (2007). Based on their studies they observed a significant increase in the levels of TEC, Hg, PCV, MEV, MEH and MEHC while TLC decreased significantly. Verma (2007) calculated the effect of lindane on the blood of minor carp, *Puntius sophore*. She observed marked reduction in RBCs and Hb concentration with a significant increase in total leucocyte count during her studies.

Gupta (2008) reported marked changes in the haematological parameters and structure as well as function of the haemopoietic organs of minor carp, *Puntius sophore* on exposure to copper. Haematological studies of fresh water fish, *Clarias batrachus* L. exposed to mercuric chloride (Hg₂Cl₂) were made by Maheswaran *et al.* (2008). They observed decline in RBC count and Hb content in metal treated groups. The number of white blood cells (WBC) however, was reported by them to increase in mercuric chloride treated groups. Tran-Duy *et al.* (2008) studied the effect of oxygen concentration and body weight on haematological parameters of Nile tilapia, *Oreochromis niloticus*. There
was an effect of fish size on haematological parameters except MCV. But there was no effect of oxygen level on any of these parameters. However as fish grow bigger, RBC, Hb and Hct became significantly reduced. There was also no interactive effect of fish size and oxygen level on any of the parameters.

Toxicological effects of leather dyes (Bismark brown and Acid leather brown) on total leucocyte count of fresh water teleost, *Cirrhinus mrigala* (Hamilton) were studied by Afag and Rana (2009). They observed leucocytosis in fishes treated with Bismark brown and Acid leather groups. Bani *et al.* (2009) evaluated the effect of constant light (24L: 0D), no light (0L: 24D) and two light dark periods (18L: 06D), (12L: 12D) on the haematological variables of juvenile great sturgeon *Huso huso*. Fish reared under a 12L: 12D photoperiod had higher haemoglobin values and erythrocyte number than those exposed to other photoperiods, while no difference could be observed between groups with regard to haematocrit value or leucocyte number. The highest survival rate (89%) was observed in the 12L: 12D period than the other two periods. Velisek *et al.* (2009) assessed the effect of an insecticide, bifenthrin on rainbow trout *Onchorhynchus mykiss* based on biochemical examination of fish. They noticed a significant decrease in plasma ammonia and increase in glucose, creatine kinase etc. A significant decrease in MCV, MCH and band neutrophil granules was observed in treated group as compared to control fishes by them. Degeneration of hepatocytes was also observed by them.

Srivastava and Choudhary (2010) studied the influence of artificial photoperiod on the blood cell indices of an Indian cat fish *Clarias batrachus* (Linn.). Total RBC count, WBC count, DLC were reported by them to remain unaffected in both the artificial photoperiod regimes (24L: 0D and 0L: 24D). However, lymphopenia and neutrophilia were observed under 24L: 0D photoperiod. Blood chloride levels were higher in 24L: 0D where as glucose levels remained unchanged in both the photoperiod treatments. Effect of lead pollution on fish Nile tilapia with special reference to haematological, immunological and biochemical parameters were assessed by Zaki *et al.* (2010a, b). RBCs, Hb, Hct and MCHC were observed by them to show significant increase. Serum GPT, GOT, LDH, glucose and cortisol also increased while serum cholesterol decreased significantly.
Impact of rearing silver carp, *Hypophthalmichthys molitrix* up to fingerling stage under three different stocking densities was investigated by Kamal and Omar (2011) upon the haematological and biochemical parameters. Fish were cultured in duplicates in cement ponds under stocking densities of 3, 6 and 9 fish/m$^3$ as T$_1$, T$_2$ and T$_3$ respectively. Results of haematological analysis showed significant increase in T$_1$ for values of RBC count, Hb, haematocrit and mean corpuscular volume (MCV) while the lowest values were recorded in T$_3$ for all three parameters.

Acute haematological response of a cichlid fish *Sarotherodon melanotheron* exposed for 96 hrs to crude oil concentration of 0, 50, 125, 250, 375 and 500mg/L of water obtained from the fish source was evaluated by Obemeata *et al.* (2012). They observed an increase in red blood cells (RBC), Hb, PCV, thrombocytes and lymphocytes in control groups while observed an increase in white blood cells, neutrophils, Leucocrit and monocytes of treated groups. Moody *et al.* (2013) examined the bioaccumulation of some heavy metals (lead, chromium and cadmium) in some freshwater fishes (*Tilapia zilli*, *Labeo cubie* and *Synodontis membranaceous*) and their haematological indices in the river manyara in Nigra state, Nigeria.

**Stressors (natural and anthropogenic) and immunology**

Snieszko (1969) studied the immune response of eurythermal species at different temperatures. They found that fishes have optimum temperature ranges below which immune response may get suppressed or even absent when maintained at low temperatures below 9°C to 12°C. Avtalion *et al.* (1970) evaluated the effect of temperature on specific immune response especially the humoral response of carps immunized against bovine serum albumin. Their studies revealed that the primary response was suppressed at low temperature.

Harris (1973) observed that dace, *Leuciscus leuciscus* are capable of producing antibodies over their complete environmental temperature range of 2-18°C. Kendall (1975) found tubular degeneration and eosinophilic, proteinaceous hyaline droplets and an increase in the amount of haemosiderin or melanin like intratubular deposits in catfish (*Ictalurus punctatus*) when exposed to methyl mercury. Antibody forming cell response in spleen, pronephros and metanephros in relation to temperature was studied by Rijkers.
et al. (1980) in carp *Cyprinus carpio*. Lowering temperature induced a delay in the peak of the primary response but had no effect on the magnitude of the response.

Starvation was observed by Aguis and Robert (1981) to result in considerably increased deposition of melanomacrophages within spleen and kidney of *Pleuronectes platessa, Salmo gairdneri, Xiphophorous helleri, Tilapia zilli* and *Scyllarhinus canicula*. They also found that catabolic tissue breakdown is a major factor contributing to the formation of the pigments within the melanomacrophages. Changes in the liver of *Tilapia mossambica* after exposure to the organophosphate monocrotophos were reported by Desai et al. (1984). Necrosis and vacuolization of hepatocytes were observed at initial stage of intoxication while fat degeneration became conspicuous in the later part of experiment.

Effect of Amitrole on the kidney tissue of Coho Salmon was observed by Rand and Petrocelli (1985) to result in necrosis of tubular and surrounding haemopoietic cells. Other changes included pyknosis and karyorrhexis of kidney tissue cells. Fries (1986) studied immunosuppression following prolonged captivity, handling, hypophysectomy, radiation, changes in temperature, salinity and exposure to chemicals including environmental pollutants. He found new evidence for immunosuppression following exposure to benzo-a–pyrene, pentachlorophenol and hexachlorobenzene.

Cossarini-Dunier (1987) observed immune responses of carp, *Cyprinus carpio* up on short term and long term exposure to effluent. He found that while short term effluent exposure caused stimulation of antibody producing cells, long term exposure resulted in the suppression of the immune system. Effect of manganese ions (MnCl$_2$) on haematocrit and the antibody response of carp, *Cyprinus carpio* were investigated by Cossarini-Dunier et al. (1988) after exposure of fish to contaminated water for 2.5 months. They noticed that contamination of water with 50mg/l Mn$^{2+}$ did not affect antibody production but significantly decreased haematocrit. Manganese ions were also observed by them to get accumulated in lymphoid organs viz. spleen and head kidney.

Maule and Schreck (1990) studied the effect of acute stress and cortisol treatment on the number of leucocytes in the blood, thymus, spleen and anterior kidney of juvenile Coho salmon *Onchorhynchus kisutch*. Numbers of leucocytes were observed by them to increase significantly in thymus and anterior kidney of acutely stressed or cortisol fed fish.
but decreased significantly in blood and spleen within one day after treatment. Tripathi and Shukla (1990) reported that liver cells from *Clarias batrachus* when exposed to methyl parathion for 7 days became loosened and showed enlargement of the hepatocytes having big nuclei and nucleoli. Their ultra structural studies on methyl parathion treated liver cells showed disappearance of glycogen granules, appearance of numerous smooth endoplasmic reticulum, lysosomal dense bodies and swollen mitochondria. These changes in the liver according to them were indicators of necrosis as a result of hepatic toxicity.

Lindane in rainbow trout *Onchorhynchus mykiss* was shown by Dunier et al. (1994) to cause a strong depressing effect on the chemiluminescent response of head kidney macrophages and a decrease of lymphocyte proliferation with B mitogens, while the number of B-lymphocytes was not modified.

Dunier (1996) evaluated the effect of pesticides such as organochloride (lindane), organophosphates (trichlorfon), insecticides and herbicides atrazine and heavy metals (Cu, Zn, Mg, Mn and Hg) on the immune system of rainbow trout and carps. All these pollutants he stressed invariably resulted in immunosuppression in all of the fresh water fishes studied by him. Tort et al. (1996) studied the effect of crowding on the immune system of sea bream *Sparus aurata*. Indicators of the immune responses they monitored were blood lymphocyte count and selected humoral responses such as the haemagglutination activity (HA) of serum towards rabbit erythrocytes (Ra RBC) and alternate complement pathway (ACP) levels. Immunodepression was indicated by decrease in ACP levels after 5 days and haemagglutination titre and circulating lymphocytes after 9 days.

Increased amount of macrophages in kidney and spleen of blue gill sunfish-*Lepomis machrocheirus* after exposure to diazinon was reported by Dutta et al. (1997). Lymphocytic cell depletion in the white pulp areas (hypocellularity) and compact fibroid networks was reported by Hart et al. (1997) in *Tilapia* on exposure to pesticide lindane.

Effect of exposure of flounder *Platyichthys flesus* to tributyltin (TBT) concentrations similar to those found in nature was studied by Grinwis et al. (1998). TBT exposure has been found to exhibit relationship to immune efficiency in fish. This was associated by them with gill lesions and reduction in the volume of thymus. Holladay et al. (1998) observed increase in vacuolation of both stromal and parenchymal cells in the
pronephros of fish, *Oreochromis niloticus* on exposure to polycyclic aromatic hydrocarbons PHA Benzo [a] pyrene.

Morvan *et al.* (1998) observed the specific and non specific immune response of fish in relation to water temperature. Their studies inferred that lower temperature adversely affects specific immune responses mediated by T-helper cells. Effect of temperature on non specific defense such as phagocytosis and cytotoxicity and total immunocompetence were also discussed by them.

Effect of environmental contaminants 2, 3, 7, 8-tetrachlorodibenzo-p-Dioxin (TCDD) on *Oreochromis niloticus* were studied by Hart *et al.* (1999). Significant reduction in total cell count in both spleen and pronephros was observed by them. Cellular depletion was evident histologically at the higher dose levels, particularly with in the lymphoid organs of the fish spleen and pronephros. It was observed by them that there is an increase in number of apoptic cells in histological preparations.

Arunkumar *et al.* (2000) evaluated the effect of trivalent (chromium chloride) and hexavalent (potassium chromate) forms of chromium in African mouth breeder *Oreochromis mossambicus* (Peters) with reference to the humoral immune response and lymphoid cells/ organs. Fatima *et al.* (2001) evaluated the effect of paper and pulp mill effluent under standard laboratory conditions on the immune system in a fresh water fish *Channa punctatus* (Bloch). They exposed fishes to 1% concentrations of paper and pulp mill effluent. Both paper and pulp mill effluent exposure was reported by them to result in a decrease in the splenic and pronephric cellularity. Effects of the length of exposure on various parameters were also studied by them. Whereas short term exposure for 15 days did not show any significant effect, long term exposure for 30, 60 and 90 days however caused significant reduction in weights of lymphoid organs viz. spleen and head kidney. Sweet and Zelokoff (2001) conducted studies to determine the relative sensitivities of fish and human cell to mercury exposure.

Alcorn *et al.* (2002) studied the effects of rearing temperature viz. 8°C and 12°C on immune functions in sockeye salmon (*Onchorhynchus nerka*). They, on the basis of their studies reported that fish reared at 8°C tend to have a greater percentage of phagocytic kidney macrophages during first two years of sampling than those reared at 12°C. However, a greater portion of the blood leucocytes were lymphocytic in fish reared
Variation in different leucocyte subpopulations were measured in photo-manipulated out of season (0+) Atlantic salmon smolt by use of flow cytometry by Melingen et al. (2002).

Ortuno et al. (2002) studied the effect of combining different stressors on innate immune responses of sea bream, Sparus aurata L. The fish were exposed to stressors viz. physical disturbances, crowding, and anesthesia with 2- phenoxy ethanol and air exposure. The results showed that physical disturbances, crowding and anesthesia produced an increase in glucose and cortisol concentrations. Crowding and anesthesia was also found to induce a depression in complement activity but hypoxia caused a reduction in the respiratory burst.

Functional alterations associated with winter syndrome in gill head sea bream, Sparus aurata were evaluated by Gallardo et al. (2003). A reduction in weight as well as haematocrit and TEC was observed in winter syndrome fishes. Red cell volume however did not show any change but the concentration of total plasma protein in winter syndrome fish was higher than in control fish, due to rise in β2 and α globulins.

Milston et al. (2003) observed the effect of short term exposure to a xenobiotic chemical o, p-dichlorodiphenyldicloro-ethylene (o, p-DDE) during early life stages on the long term immune competence of Chinook salmon Onchorhynchus tshawytscha. Immersion of Chinook salmon eggs in a nominal concentration of o, p-DDE for 1 hour at fertilization followed by immersion in the same dose for 2 hours at hatch resulted in a significant reduction in the ability of splenic leucocytes from fish 1 year after treatment. Miwa et al. (2003) studied the impairment in the development of thymus in cultural osmerid fish, the ayu, Plecoglossus altivelis.

Barcellos et al. (2004) exposed jundia (Rhamdia Quelen quoy and Gaimard Pimelodidae) to acute and chronic stress usually encountered during aqua cultural management so as to visualize its effects on immune system. The acute stress was induced by draining the water tank and transferring fish to another tank while chronic stress was induced by maintaining fish in crowded concrete tanks. It was observed that lymphocyte count observed reduction of approximately 80%. This reduction they stated decreased the ability of fish to mount an immune response to environmental pathogen. To evaluate the impact of delayed initial feeding on the development of immune system of
*Oreochromis niloticus*, Tiongco *et al.* (2004) employed histological techniques on them. They observed a general decrease in the overall size of the fish as well as the immune organs they studied when the initial feeding of fish was delayed for four days. This they stated makes fish more susceptible to diseases.

The hazardous effect of the pyrethroid insecticide fenvalerate on the histology of the liver of the catfish *Clarias gariepinus* after exposure to 1/10 LC$_{50}$ for 5 and 10 days was investigated by Sarkr and Allail (2005). The results showed that the changes induced in the liver included cytoplasmic vacuolization of the hepatocytes, blood vessel congestion, inflammatory leucocyte infiltration, necrosis and fatty infiltrations. Alterations in the immunological parameters of tench *Tinca tinca* after acute and chronic exposure to lethal and sublethal treatments with mercury, cadmium and lead were studied by Shah and Altindag (2005). Both acute and chronic metal exposure they stated caused immunological impairment and thereby weakens the immune system of fish and in turn increased their susceptibility to infections. Witeska (2005) stated that even short term exposure to heavy metals induces a persistent stress in fish. This, they stated seemingly render more susceptible to diseases.

Khoshbavar-Rostami *et al.* (2006) assessed the lysozyme activity, chemiluminiscense response and immunocompetent cell population size in great sturgeon *Huso huso*. The WBC count and lymphocyte count in fish exposed to diazinon were observed to be significantly lower than control groups while the level of neutrophils was higher. However, no significant differences were observed by them in the values of monocytes, eosinophils and immature neutrophils between these two groups. Fish, *Liza persia* was exposed to DDT (acute, 0.1ppm) for 15 days by Pandey *et al.* (2006) to assess its toxicity. Exposure to DDT was found by them to induce dilation of blood sinusoids, vacuolization and granular degeneration of hepatocytes. Complete necrosis of hepatocytes at places (focal necrosis) and fibrosis were noticed on day 15 of the experimental period. Blood sinusoids also depicted complete disorganization toward the end of the experiment.

Velmurugan *et al.* (2007) studied the effect of fenvalerate on different freshwater fish, *Cirrhinus mrigala*. Necrosis of tubular epithelium, pyknotic nuclei in the haemopoietic tissue, hypertrophied epithelial cells of renal tubules, narrowing of the
tubular lumen, expansion of space inside the bowman’s capsule and contraction of the glomerulus were observed in the kidney tissue of the fish by them.

Bowden (2008) reviewed the modulation of immune system of fish and states that increase in light, temperature, salinity, oxygen, pH or particulates results in general increase in immune functions. Lepak and Kraft (2008) subjected forage fish Alewife, *Alosa pseudoharengus* to contrasting cold temperatures of 4°C and <2°C representing mild and severe winter conditions. Circulating lymphocytes from Alewife exposed to severe winter temperature were significantly lower in number (40%) compared to those who experienced milder winter conditions. Oshode (2008) assessed the toxic effects of raw leachate obtained from the Aba-Eku landfill (AERL) on *Clarias batrachus*. Histopathological lesions they added were concentration dependent and well marked in the gills, kidneys and liver of exposed fishes. Saxena and Saxena (2008) used polluted water from a brook to investigate the effect of heavy metals on fish lymphoid organs.

Freshwater fish, *Channa punctatus* was exposed to sublethal concentration of chloracetanilidae herbicide alachlor technical grade and lasso 50% EC for a period of 10 days by Butchiram *et al.* (2009). The tissue damages like degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture of blood vessels and deposition of hepatic cords were the changes observed in the liver. The changes in the kidney included necrosis, swelling of renal tubules, cellular hypertrophy and granular cytoplasm Suresh (2009) studied the toxic effect of a heavy metal cadmium chloride on the occurrence of melanomacrophage centres (MMCs) of liver, spleen and kidney of *Tilapia mossambicus*. The fish following median lethal concentration of cadmium chloride for 120 hours were observed by him to depict that average number and size of melanomacrophage centres (MMCs) significantly increased compared to control fishes.

Effect of lead, mercury and cadmium on both humoral and cellular immune system of *Oreochromis niloticus* was carried out by Aboud (2010). Based on his studies he stated that lead, mercury and cadmium have inhibitory effect on the cell mediated immune system as an inhibitory effect was observed on the phagocytic activity of fish. Effect of these metals on humoral immune system too was found to exhibit inhibitory effect as revealed by low levels of antibodies and high mortality rates in fish. The effect of short term starvation on some haematological, biochemical and non specific immune
response parameters together with the histological structure of skin were investigated in European eel *Anguilla anguilla* by Caruso *et al.* (2010). They found that starvation for 58 days did not affect haemoglobin and haematocrit value but there was an increase in glucose and cortisol levels in fish after 42 days of starvation. Haemolytic and haemagglutinating activities were reported by them to decrease in starved eels. Ghiasi *et al.* (2010) studied the effects of low concentrations of cadmium on fish’s innate immune system during low temperature seasons.

Jiang *et al.* (2012) exposed freshwater fish *Carassius auratus* gibelio var to different concentrations (0.1, 0.2, 0.5, 0.8 and 2.0 mg/L) of Cu for 96 hrs and observed the acid phosphate (ACP) and alkaline phosphate (ALP) activities from the kidney, liver, gill, spleen, muscles and brain. They observed that at highest concentration of Cu (2.0mg/L) ACP activity decreased significantly in kidney, liver, gill and spleen but increased significantly in muscle and brain. Kreutz *et al.* (2012) studied the immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure (24hrs) to sublethal concentration of glyphosate. They observed significant reduction in phagocytic index, serum bacteria agglutination and total peroxidase.

**Stressors (natural and anthropogenic) and reproductive cycle**

Preliminary observations on the effects of temperature and light upon reproduction in *Gambusia affinis* were made by Medlen (1951). Vlaming (1971) observed the effects of food deprivation and salinity changes on reproductive functions in Estuarine Gobid fish, *Gillichthys mirabilis*. Ueda and Takahashi (1977) reported that mature male and female gold fish, *Carassius auratus* when treated with clomiphene citrate at a dose of 1µg/g body weight every third day for 30 days showed significant advance in gonadal development.

Effect of insecticidal pollution on ovarian recrudence in the teleost fish *Channa punctatus* was studied by Saxena and Garg (1978). They observed degeneration of oocytes and reduction in the ovarian growth. Effect of mercuric chloride on the reproductive cycle of the teleostean fish *Channa punctatus* was determined by Ram and Sathyanesan (1983) and observed atretic follicles and degeneration of stage I and II oocyte in the ovary of treated fishes.
Ram and Sathyanesan (1986) studied the effects of a mercurial fungicide on the gonadal development of the teleostean fish *Channa punctatus* (Bloch.). They exposed fish to a safe concentration of 0.20 ppm of a commercial fungicide, Emisan (MeEHgCl) for 6 months from January to June. Significant inhibition of gonadal development was depicted by their histology and reduced gonadosomatic index. Degenerative changes in oocytes were also observed by them.

The development of rainbow trout ovaries and changes in plasma vitellogenin and Ca\textsuperscript{2+} concentration was investigated for two annual photoperiod cycles, one advanced and another delayed for 1.5 months by Nakari and Pesonen (1988). Ovarian development, increase in plasma vitellogenin and total calcium concentration was significantly faster in advanced than in delayed and control groups of fishes. The honmoroko (*Gnathopogon caerulescens*), a small, spring-summer breeding cyprinid fish was exposed to various temperature and photoperiod regimes during different phases of its annual reproductive cycle by Okuzawa *et al.* (1989). The gonadosomatic index, plasma gonadotropin (Gt.H) and gonadal steroids were higher in fish held at long photoperiod (15L/9D) than in those held at short photoperiod (12L/12D). During its spawning season a long photoperiod (15L/9D) was again required to maintain gonadal activity and high plasma Gt.H and gonadal steroid levels, especially at high temperature(25°C).

Nath and Kumar (1990) investigated the impact of nickel on the histological architecture of gonads of both sexes of a fresh water tropical perch, *Colisa fasciatus*. Marked degenerative alterations were observed in both testes and ovaries. General disorganization in the testicular lobules and comparatively large interfollicular space was observed by them. Several oocytes were observed in the process of absorption exhibiting atresia. Kime (1995) studied the effects of pollution on reproduction in fish. Adult male and female *Oreochromis mossambicus* were analysed by Carnish *et al.* (1996) for gonadal mass and length, gonadosomatic indices, blood pH and gonad pH over a period of twelve month experimental period. They observed an inverse relationship between blood and gonadal pH.

Baruah and Das (2002) studied the histopathological changes in the ovaries of fish, *Heteropneustes fossilis* on exposure to paper mill effluent. Significant atresia in the
ovary with major damage to younger oocytes was observed. They also noted partial lysis, swelling atresia and changes in position of nucleus.

Effect of in vivo exposure to pp-DDE and dieldrin on seasonal gonadal development and steroidogenesis in female largemouth bass, *Micropterus salmoides floridanus* was studied by Muller *et al.* (2002). They observed a decrease in estradiol production but no change in 11-keto testosterone levels on exposing to high concentration of pp-DDE and dieldrin. Moreover, no change in GSI or LSI was observed under treatment of dieldrin or pp-DDE regardless of dose.

Effects of copper toxicity on growth, gonadal development and reproductive performance were studied for 140 days by James *et al.* (2003). They observed decrement in the rates of food intake, food conversion and conversion efficiency. Reproductive indices like gonad weight, gonadosomatic index and fertility also exhibited significant reduction in relation to copper concentration.

Dutta and Maxwell (2003) studied the effect of sublethal concentration (60µg/l) of insecticide diazinon (an organophosphorus compound) on histological changes in ovaries of blue gill (*Leopomis macrochirus*) at 24, 48, 72 and 96 hour and 1, 2 and 3 week interval. After 24 hours, oocyte II showed adhesion and cytoplasmic retraction and cytoplasmic degeneration. Additional adhesion and more retraction were observed at 48 hours in oocyte II. He further noted that at 72 hours whereas adhesion retraction and cytoplasmic expulsion of oocyte IV became more intense after 96 hours exposure.

Effect of methylmercury on egg and juvenile viability in two populations of killifish, *Fundulus heteroclitus* was determined by Khan and Weis (2005). They exposed eggs of killifish from a polluted creek, Piles creek (PC) and a relatively pristine estuary in long island (LI) to various concentrations of methylmercuric chloride (MeHg) prior to combination with untreated sperm. They observed that LI killifish eggs were less tolerant to MeHg than PC eggs.

To evaluate the effects of long term hypoxia on reproduction, Gulf killifish were subjected to either normaxia (6.68±2.1mg/L DO) or hypoxia (1.34±0.45mg/L DO) for one month by Landry *et al.* (2007). Fecundity, growth, gonadosomatic index (GSI), circulating sex steroids (testosterone, T; 11-ketotestosterone, 11KT and estradiol 17β, E2)
and egg yolk protein (vitellogenin, VTG) measured. Hypoxia was observed by them to significantly reduce growth and reproduction. E2 was 50% lower in females and 11 KT was 50% lower in males, although the precursor hormone T remained unchanged in either sex after hypoxic treatment.

Influence of pesticide endosulfan (an organochloride compound) on the ovaries of blue gill fish, *Lepomis macrochirus* were studied by Dutta and Dalal (2008). Exposure for 24 hours with histological preparations at 25% (0.25µg/L), 75% (0.75µg/L) and 100% (1µg/L) sublethal concentrations were examined. The control contained an abundance of the different stages of oocytes (oocytes I, II, III and IV) and had an intact ovigerous lamellae and follicular lining. After 24 hours exposure to 25% concentration most of the oocytes of II and III stages were observed to show damaged stroma and cytoplasmic retraction.

Ternitope and Oyedopo (2008) evaluated the effect of neem *Azadirachta indica* on the histology of gonads in *Tilapia zilli* (Gervais). They observed degeneration of stage I and II oocytes. Moreover considerable increase of atretic follicles was observed by them.

Impact of pollution on the histopathological structure of gonads in *Oreochromis niloticus* from Rosetta Branch, Nile river, Egypt was evaluated by Mazrouh and Mahmoud (2009). They observed that fish exposed to higher concentration of pollutants showed higher incidence of gonadal abnormalities in the form of deformed oocytes and spermocytes. They also observed reduction in the number of oocytes and spermocytes besides the arrest of process of oogenesis and spermatogenesis.

Rudolf (2009) evaluated the effects of hypoxia on fish reproduction and development. Results showed that hypoxia had profound effect on fish reproduction and development. Both laboratory and field studies evidently showed that hypoxia can cause major reproductive impairment by inhibiting testicular and ovarian development, therefore exerting a negative impact on production and quality of sperm and egg. He further reported that fertilization and hatching success also affects larval survival as well as the quality and fitness of juveniles. Sarosiek (2009) studied the effect of copper, zinc, cadmium and mercury ions (100, 10 and 1mg/l) on the activity of some enzymes of carp, *Cyprinus carpio* L. spermatozoa.
Impact of neem oil on ovarian changes in freshwater fish *Glossogobius giuris* was determined by Naranaswamy and Ramachandra (2010). They exposed fishes to various concentrations of neem oil (0.05ppm, 0.25ppm and 0.50ppm) for 24, 48, 72 and 96 hours. Lower dosage (0.05ppm) of neem oil for 24 hours recorded an increase in number of yolk vesicles and degeneration of oocyte I and II with a reduction in ovarian weight. A higher dosage (0.50ppm) of neem oil for 72 and 96 hours resulted in an increase in number of atretic follicles. While liquification, swelling of follicular wall, ruptured follicles and necrosis was also observed at higher dosage. Singh *et al.* (2010) noticed testicular regression in *Channa punctatus* maintained under long photoperiod at warm temperature (LD 16: 8-30°C) and short photoperiod at warm temperature (LD 8: 16-30°C).

Hypoxia was observed by Lo *et al.* (2011) to exhibit changes in primordial germ cell (PGC) migration in Zebra fish (*Danio rerio*) embryo. They stated that embryo showed PFC migration defect as indicated by the presence of mis-migrated ecotopic PGCs. The impact of polluted water from the Egyptian Eastern Mediterranean coast on reproductive, toxicological and haematological characteristics of *Siganus rivulatus* was determined by Moharram *et al.* (2011). Erythrocyte number, haemoglobin content and packed cell volume showed a decrease while leucocyte number exhibited an increase indicating hypochromic macrocytic anemia and susceptibility of fish to infection and stress. Testosterone and progesterone levels decreased in females, while in males progesterone level increased.

Sharma *et al.* (2011) evaluated sublethal effect of cadmium chloride in the ovaries of the air breathing fish, *Heteropneustes fossilis*. They exposed fishes to sublethal concentrations of 6mg/l and 9mg/l for 15, 30 and 45 days. While after 15 days of 6mg/l exposure ovaries showed enlarged oocytes and degeneration of egg envelope was evident by 30 days. 45 days exposure however, resulted in appearance of atretic follicles with scattered nuclei. In 9mg/l concentration after 15 days, ovarian wall was observed to be slightly thinner and ruptured whereas enlarged intrafollicular spaces were permanently seen after 30 days. Egg envelopes however, got degenerated following 45 days exposure to cadmium chloride. Ramsden *et al.* (2013) studied the sublethal effects of titanium dioxide nanoparticles on the physiology and reproduction of Zebrafish.