General haematological and immunological profile of *Garra gotyla gotyla*

Studies on the general haematological parameters viz. TEC, Hb, Hct, MCV, MCH, MCHC, TLC, DLC of fish *Garra gotyla gotyla* has been undertaken in order to establish the reference values/normal range of these parameters. General immunological profile of fish *Garra gotyla gotyla* has also been established by studying the immune organs like thymus, spleen and anterior kidney. These reference values of haematological
parameters and immunological parameters help to assess the health status of fish in natural conditions as well as in laboratory conditions.

**Seasonal variations in haematological parameters of fish Garra gotyla gotyla**

During present studies, seasonal variations in haematological parameters of *Garra gotyla gotyla* have been investigated for a period of two years from November 2009 to October 2011 from Jhajjar stream. During this period, seasonal changes in temperature and dissolved oxygen have been monitored so as to assess how these influence the various haematological parameters.

RBC dependent parameters viz. TEC, Hb and Hct exhibited increase during spring and summer seasons with an increase in water temperature and decline in DO content of Jhajjar stream and observed maxima during summer season (June). From here onwards a significant decline in the values of these parameters have been observed during monsoon through autumn till winter with corresponding decline in water temperature and increase in DO content and witnessed minima during winter (January).

Presently a positive correlation has been noticed between RBC dependent parameters and temperature, whereas DO and RBC dependent parameters exhibit a negative correlation. During spring and summer with increase in water temperature DO retaining capacity of water decreases and therefore a significant rise in values of TEC, Hb and Hct have been observed. Afterwards, during monsoon, autumn and winter DO retaining capacity of water increases with decline in water temperature and hence significant decline in the values of these parameters is justifiable.

Besides temperature, other important factors that influences the RBC dependent parameters are photoperiod, feeding intensity and breeding period. During spring and summer, with increase in day length, an increase in photoperiod has been observed which ultimately results in an increase in haematological parameters as well as feeding regime in *Garra gotyla gotyla*. Temperature also influences the feeding activity and metabolic rate of fish. Presently June and July have been observed to be the breeding period of fish *Garra gotyla gotyla*. Thus to meet increased energy demand during this period, (breeding period) fish exhibit an increment in various RBC dependent parameters.
Calculated indices of RBC include mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), where MCV and MCH recorded their peak during winter (January) as they showed an increasing trend in their values during monsoon through autumn till winter. Contrary to MCV and MCH, MCHC exhibit maxima during summer (June).

Total leucocyte count (TLC) and differential leucocyte count (DLC) including both agranulocytes (monocytes and lymphocytes) and granulocytes (eosinophils, basophils and neutrophils) exhibited an increment during spring and summer while all these parameters depict a decline during monsoon, autumn and winter. Thrombocytes, one of important constituent of DLC, however, exhibited increase during monsoon through autumn till winter season. Thus an increase in the values of TLC and DLC seem to be an immunostimulatory response on the part of fish to combat the temperature related stress.

**Seasonal variations in the cortisol levels of *Garra gotyla gotyla***

Presently cortisol hormone has been estimated during the five main seasons viz. spring, summer, monsoon, autumn and winter. Cortisol has been observed to exhibit smooth increase during spring and summer season. After attaining peak during summer (May), it has been observed to undergo gradual decline with the corresponding declining temperature and touches its lowest ever value in winter (January).

Presently it has been observed that temperature related stress, possibly have resulted in highest level of cortisol during spring and summer. During this very period decreasing DO content due to increased rate of evaporation (because of increase in temperature) from waterbody exert a sort of stress on fish. In order to compensate the temperature dependent availability of oxygen, fish *Garra gotyla gotyla* therefore exhibit an increase in cortisol titre.

Increment in cortisol titre during spring and summer also finds a direct relation with feeding intensity. Its increase during this period also has a direct association with breeding period of fish which extends from June to July. During this very period vitellogenic activity geared up which happen to be most energy demanding process in fishes. In order to fulfill this energy demand, therefore, fish *Garra gotyla gotyla* exhibit
an increase in its cortisol titre which possibly resulted in release of more and more glucose (the energy currency of body).

From monsoon onward, till winter, a decline in water temperature has been observed which result in lowering down the secretion of cortisol hormone since during this period, fish does not need any extra energy for gonadal development as the most energy demanding period is over.

**Seasonal variations in spleen somatic index (SSI) of Garra gotyla gotyla**

Significant variations have been observed in the spleen somatic index (SSI) of fish *Garra gotyla gotyla* throughout the year. A gradual decrement in the values of SSI has been observed during the spring and summer season and afterwards a significant increment in the values of SSI has been noticed during monsoon till winter. Decline in SSI during spring and summer finds a direct relation with increase in GSI of fish during this period which is the breeding period of fish. During breeding period physiological adjustments were made in different systems including immune system in order to provide maximum energy for the development of gonads and hence decrement in SSI was observed. Moreover to overcome temperature related stress fish contracts its spleen and release more and more leucocytes in general blood circulation.

**Ovarian Cycle of Garra gotyla gotyla:**

Fish under present investigation, *Garra gotyla gotyla* has been observed to have a pair of ovaries lying ventral to kidneys and covered dorsally by a thin black peritoneum. Histological observations of ovaries revealed that these were bounded by an outer covering, tunica albuginea beneath which was present germinal epithelium and a large number of oocytes at different stages of maturity were observed in the ovocoel. Based on the sequence of changes an oogonium underwent, seven oocytes stages viz. oocyte I stage, oocyte II stage, oocyte III stage, oocyte IV stage, oocyte V stage, oocyte VI stage and oocyte VII stage were recognized in the ovarian cycle. Based on the present observations, it has been deduced that:

- the ovarian activity of *Garra gotyla gotyla* follows a cyclic pattern and can be divided broadly into six phases viz. immature/ virgin phase (September-October), early maturing phase (November-January), developing phase
February-March), developed phase (April-May), spawning phase (June-July) and spent/ resting phase (August).

- fish shows asynchronous development of oocytes.
- fish falls under the category of monsoon spawners.
- fish possess a short spawning period of two months, from June to July.

**Haemato-immunological variations during the reproductive cycle of *Garra gotyla gotyla***

Both haematological (TEC, Hb and Hct) as well as immunological (spleen somatic index and total leucocyte count) parameters exhibit significant variations during different phases of reproductive cycle in females of *Garra gotyla gotyla*. From the present observations on haematological parameters (TEC, Hb and Hct), it is evident that the values of TEC, Hb and Hct witness increase during the developing, developed and spawning phase of reproductive cycle. This increase in values of RBC dependent parameters viz. TEC and Hct during these phases finds a direct relation with increase in water temperature. Presently, cortisol level revealed a significant increase (P<0.01) in its values during breeding period and thus confirms that the fishes are under stress during heightened reproductive period.

Similar to RBC dependent parameters, total leucocyte count which is the main defense parameter of fishes finds a great usage in monitoring the health/ immune status of fish. TLC has been observed to depict an increase during the developing, prespawning and spawning phase of reproductive cycle. This increase in TLC values finds a direct relation with the feeding status of fish. During this period females feed voraciously to meet the energy demand for the development of oocytes.

Besides haematological parameters, spleen somatic index (SSI) has also been studied as an index of health/ immune status of fish. The spleen is a major antibody producing organ in teleost fish. The decreased size of spleen and consequently the low values of SSI indicate the poor condition of fish during developing, prespawning and spawning phase. During this period it appears, spleen contracts and releases more and more leucocytes in general blood circulation. Increase in leucocyte number in general
blood circulation is a mechanism on the part of fish to make them immunologically strong to counter such stressful conditions. Further SSI of fish has been found to show a negative correlation with GSI and therefore, during this period when GSI showed an increase in its values, SSI depicted a consequent decline in its values.

Following spawning, fishes enter into spent phase and then pass through immature and early maturing phase. During this period a significant (P<0.01) decrease in the values of RBC dependent parameters has been observed. Similar to TEC and Hct, cortisol too follows the similar trend in their values during these phases of reproductive/ovarian cycle and hence observed significant decline (P<0.01). Significant (P<0.01) decline in total leucocyte count during spent, immature and early maturing phase has been recorded in all females of Garra gotyla gotyla. It is the time when SSI showed a significant (P<0.05) increase in its values and touches peak in December (early maturing phase).

**Effect of stressors**

**Anthropogenic stressor (heavy metal manganese)**

**Effect of manganese on haematological parameters of fish Garra gotyla gotyla**

Fish Garra gotyla gotyla exhibit significant (P<0.01) decline in its RBC dependent parameters viz. TEC, Hb and Hct in all the manganese treated groups (20%, 40% and 60%) during the experimental period of 9 weeks. Calculated indices viz. MCV, MCH and MCHC depicted significant alterations during this period in all metal treated groups. MCV and MCH exhibited significant increase in all concentration groups (20%, 40% and 60%) whereas MCHC showed fluctuating pattern.

Presently fish Garra gotyla gotyla has been diagnosed to suffer from **Megaloblastic anemia** under the stress of anthropogenic stressor (heavy metal, manganese) as is depicted by reduction in values of TEC, Hb and Hct in the general blood circulation.

TLC depicted significant increase (P<0.01) in all fishes following an exposure to manganese during the period of 9 weeks. Lymphocytes, monocytes and eosinophils register an increase during this period whereas neutrophils, basophils and thrombocytes
exhibit a decline in their count. Therefore lymphocytes, monocytes and eosinophils were the only cells which evoke an immunostimulatory response in fish *Garra gotyla gotyla* on subjection to metal manganese.

**Effect of manganese on haemopoietic organs of fish *Garra gotyla gotyla***

*Garra gotyla gotyla* exposed to various sublethal concentrations of manganese have shown considerable alterations in the histological details of all haemopoietic tissues, viz., liver, head kidney and spleen. Liver of fish *Garra gotyla gotyla* exhibited necrosis of hepatocytes, distension of sinusoids, vacuolation of hepatocytes, increase in melanomacrophage centres (MMCs), degenerative cellular architecture and total degeneration of tissue at the end of experimental period. Anterior kidney depicted necrosis of haemopoietic tissue, vacuolation of renal tubules, loss of integrity of renal tubules, reduction in haemopoietic tissue and total degeneration of renal and haemopoietic tissue. Spleen also exhibited degenerative changes including necrosis, vacuolation, appearance of haemosiderin pigment, proliferation of melanomacrophage centres.

All these degenerative changes in haemopoietic organs viz. liver, spleen, head kidney and ultimately affect the haemopoietic machinery of these organs which is evident from the drastic changes observed in the values of TEC and its related parameters following an exposure to manganese.

**Effect of manganese on immune Organs of fish *Garra gotyla gotyla***

Imprints of head kidney and spleen of all metal treated groups (20%, 40% and 60%) register a significant increase in the population of lymphocytes, monocytes and eosinophils during the experimental period of 9 weeks. Other constituents of lymphocytic machinery viz. neutrophils, basophils and thrombocytes depicted a decline in their number during the experimental period.

Immune organ thymus depicted significant alterations including necrosis, vacuolation, lymphocytic infiltration and total degeneration of thymic tissue.

**Effect of manganese on the ovaries of fish *Garra gotyla gotyla***
Presently significant histopathological alterations were observed in the ovaries of *Garra gotyla gotyla* when exposed to different sublethal concentrations of manganese (20%, 40% and 60%) for an experimental duration of 9 weeks. The prominent changes include a decrease in size and number of oocytes of stage I, stage II and stage III, increase in interfollicular distance, vacuolation, necrosis, increase in number of atretic follicles and at the end of experimental period a great reduction was observed in the number of functional oocytes of different stages.

Presently it has been observed that effect of manganese is time and dose dependent. Low doses compared to higher ones have been observed to produce well demonstrated effect on frequency of occurrence of different oocytes.

**Effect of manganese on cortisol levels of fish *Garra gotyla gotyla***

Under the treatment of manganese (20%, 40% and 60%) the cortisol exhibit a biphasic response i.e. initially, an increment was observed (upto 4th week) followed by a continuous decline till the end of experimental period. From 4th week onwards although cortisol level declined but never reached to normal values. This biphasic response by cortisol (primary response) also stimulates the secretion of glucose as a secondary response, which in tune with cortisol first exhibit increase in its titre upto 4th week followed by a decline upto 9th week of experimental period.

Thus increased level of cortisol by creating hyperglycemic conditions seemingly helps fishes to fight/ overcome the stressful conditions by making more energy available to them.

**Natural stressor (starvation)**

**Effect of starvation on haematological parameters of fish *Garra gotyla gotyla***

RBC dependent parameters viz. TEC, Hb and Hct have been found to exhibit significant decline (P<0.01) in starved fishes during the experimental period of 9 weeks. Among calculated indices, MCV exhibited significant increase (P<0.01) whereas MCHC depicted gradual decline in their values. MCH observed fluctuating pattern during the entire experimental period. Presently fish *Garra gotyla gotyla* has been diagnosed to
suffer from **Hypochromic macrocytic anemia** due to reduction in the values of MCH and MCHC and increased values of MCV.

TLC showed a significant decrement in their values during the 9 week experimental period. Concerning DLC a decline in some of its components like lymphocytes, monocytes, eosinophils and basophils has been observed while other constituents of DLC neutrophils and thrombocytes depicted an appreciable increase during the entire period of starvation.

**Effect of starvation on haemopoietic organs of fish *Garra gotyla gotyla***

Presently significant histological alterations were recorded in the haemopoietic organs (liver, head kidney and spleen) of fish *Garra gotyla gotyla* following exposure to starvation. Liver of starved fishes exhibited marked histopathological alterations which include necrosis, distension of sinusoids, vacuolation and degeneration of cellular architecture of liver tissue. Various histological changes including tubular vacuolation, necrosis, degeneration of haemopoietic tissue and finally loss of integrity of renal tubules were observed in head kidney whereas appearance of haemosiderin pigment (HP), necrosis (N), vacuolation (V) and total degeneration of haemopoietic tissue were the prominent changes that were observed in spleenic tissue.

Presently starvation because of non availability of essential nutrients result in degeneration of normal cellular architecture of the haemopoietic organs to such an extent that their functional capacity gets hampered.

**Effect of starvation on immune organs of fish *Garra gotyla gotyla***

*Garra gotyla gotyla* exhibit significant decline in the number of agranulocytes (lymphocytes and monocytes) and granulocytes (eosinophils and basophils) during the experimental period whereas neutrophils depicted considerable increase in their number during the entire period of starvation of 9 weeks. Thrombocytes too were observed to exhibit appreciable increase in their number.

Thymus of starved fish exhibited various histopathological changes including necrosis, vacuolation, loss of normal cellular architecture and degeneration of thymic tissue during the experimental period.
On the basis of observed alterations in leucocyte count in spleen and head kidney and histopathological observations in the thymic tissue it can be stated that starvation has resulted in the immunosuppressive state in fish *Garra gotyla gotyla*.

**Effect of starvation on ovaries of fish *Garra gotyla gotyla***

Ovaries of fish, *Garra gotyla gotyla* on exposure to starvation for a period of 9 weeks exhibit various histological alterations including contraction of oocytes and increase in interfollicular space, increase in atretic follicles and higher percental decline in number of oocytes of stages stage II and stage III compared to stage I.

**Effect of starvation on cortisol levels of fish *Garra gotyla gotyla***

Starved fishes exhibited a significant increase in their cortisol level but only upto 5th week of experimental period and afterwards a significant decline in level of cortisol has been observed during rest period of experimental duration of 9 weeks. It is noted that although the titre of cortisol starts declining after 5th week but it never restored its basal level.