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JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Seasonal changes in selected immune response of *Mystus gulio* and *Mystus vittatus*

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Publication Info

Paper received:
16 February 2011

Revised received:
20 August 2011

Re-revised received:
19 December 2011

Accepted:
17 March 2012

Abstract

Estuaries are considered as highly potential area for that including feeding, spawning and nursery rearing of most of the finfishes and shellfishes. In the present investigation, two species of catfish (*M.gulio* and *M.vittatus*) were selected to study the impact of season on their immune organs (kidney and spleen) and selected immune response from Vellar estuary. The physico-chemical parameters (pH, temperature, salinity and rainfall) were measured during the sampling period 2009-10. The non-specific immune parameters (WBC count, lysozyme activity and NBT assay) were analyzed. The immune organs (head-kidney and spleen) variation was also observed by histological studies. Our results stated that the WBCs count of *M. gulio* and *M. vittatus* increased during summer (10.3 and 10.1 X 10^6 ml^{-1}) season and decreased in monsoon (6.8 and 7.0 X 10^6 ml^{-1}). In contrast, lysozyme activity was highest in post monsoon (1540 and 1525 U min^{-1} ml^{-1}) and lowest in summer (1000 and 960 U min^{-1} ml^{-1}). The activity of NBT was highest in monsoon (0.68 and 0.65 at 540 nm) and lowest in summer (0.012 and 0.2 at 540 nm). The histological observation implies that the cell variations were different in respect to different seasons.

Key words

Immune response, Seasonal changes, *Mystus gulio*, *Mystus vittatus*

Introduction

Estuaries are the important seed collection center for most of the aquaculture activities. The fluctuation of physico-chemical characteristics in estuarine environment has a profound influence on the seasonal occurrence of the juveniles and adult fish stocks (Brinda *et al*., 2010). Catfishes are distributed all over the world, and are often used in the diet (Nobuya *et al.,* 2002). Both *Mystus gulio* and *Mystus vittatus* are the native catfish of family Bagridae distributed throughout the Indian subcontinent especially in estuarine and tidal waters and they are important target species for small-scale fishermen, who use a variety of traditional fishing gears (Begum *et al.,* 2008; Ravindra and Thilina, 2010). This small indigenous fish contain high nutritional value in terms of protein, micronutrients, vitamins and minerals which are not commonly found in other foods making it a highly favorable candidate for aquaculture in the South East Asia (Ross *et al.,* 2003).

Seasonal variations, particularly environmental temperature, have been greatly influencing the immune system of fish (Zapata *et al.,* 1992). It is well known that the higher environmental temperatures enhancing the immune responses, whereas lower temperatures adversely affect their expression (Daggfeldt *et al.,* 1993). The measurements of nonspecific immune parameters are useful as markers of pollution to determine the health status of fishes and also many cells (in particular leucocytes and macrophages) and their products (myeloperoxidase, superoxides, lysozyme, complement, acute phase proteins, interferons, agglutinins, properdins and lysins) contribute to the general immunological defence mechanism (Sahoo *et al.,* 2005).

Two species of *Mystus* varieties (*Mystus gulio* and *Mystus vittatus*) were selected in this study to examine the seasonal changes (summer, pre monsoon, monsoon and post monsoon) on immune head-kidney and spleen organs and selected immune responses from Parangipettai area.

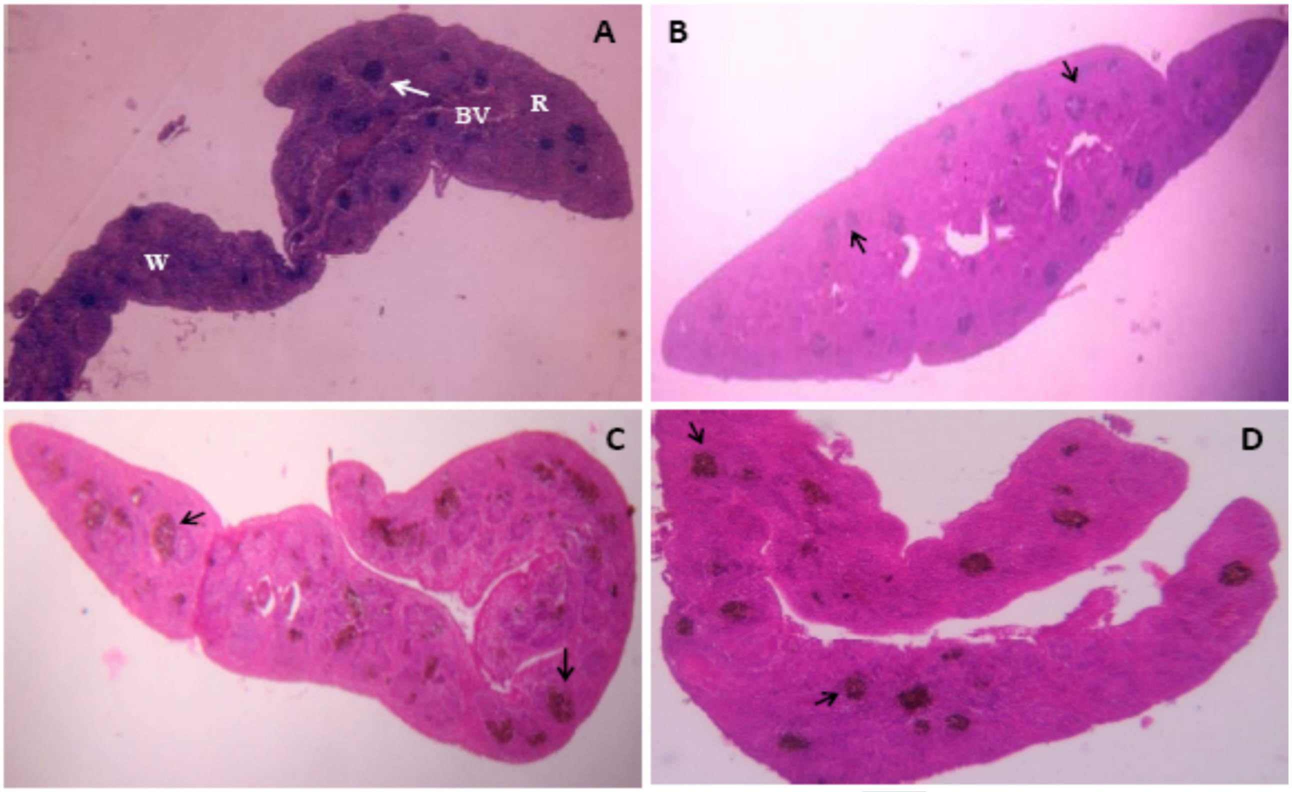

Fig. 1 : Light micrographs (100x) of spleen of *M. vittatus* in (A) Summer, (B) Pre monsoon, (C) Monsoon, (D) Post monsoon seasons. Section shows red pulp (R), white pulp (W), blood vessels (BV) consisting of elastic fibers and melano macrophage centers (MMCs)[arrows]

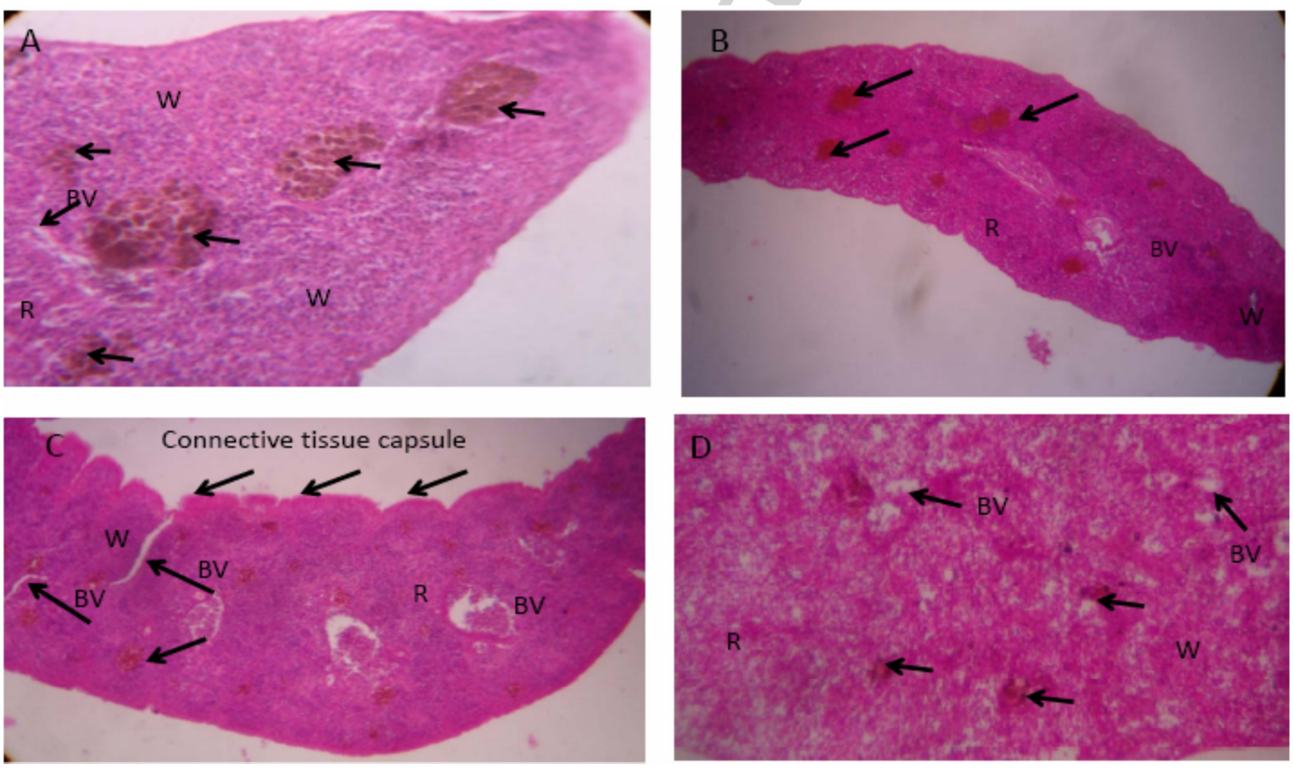

Fig. 2 : Light micrographs (600x) of spleen of *M. gulio* during different seasons. (A) Summer; (B) Pre monsoon; (C) Monsoon; (D) Post monsoon. Section shows red pulp (R), white pulp (W), blood vessels (BV), melano macrophage centers (MMC) [arrows] and connective tissue capsule

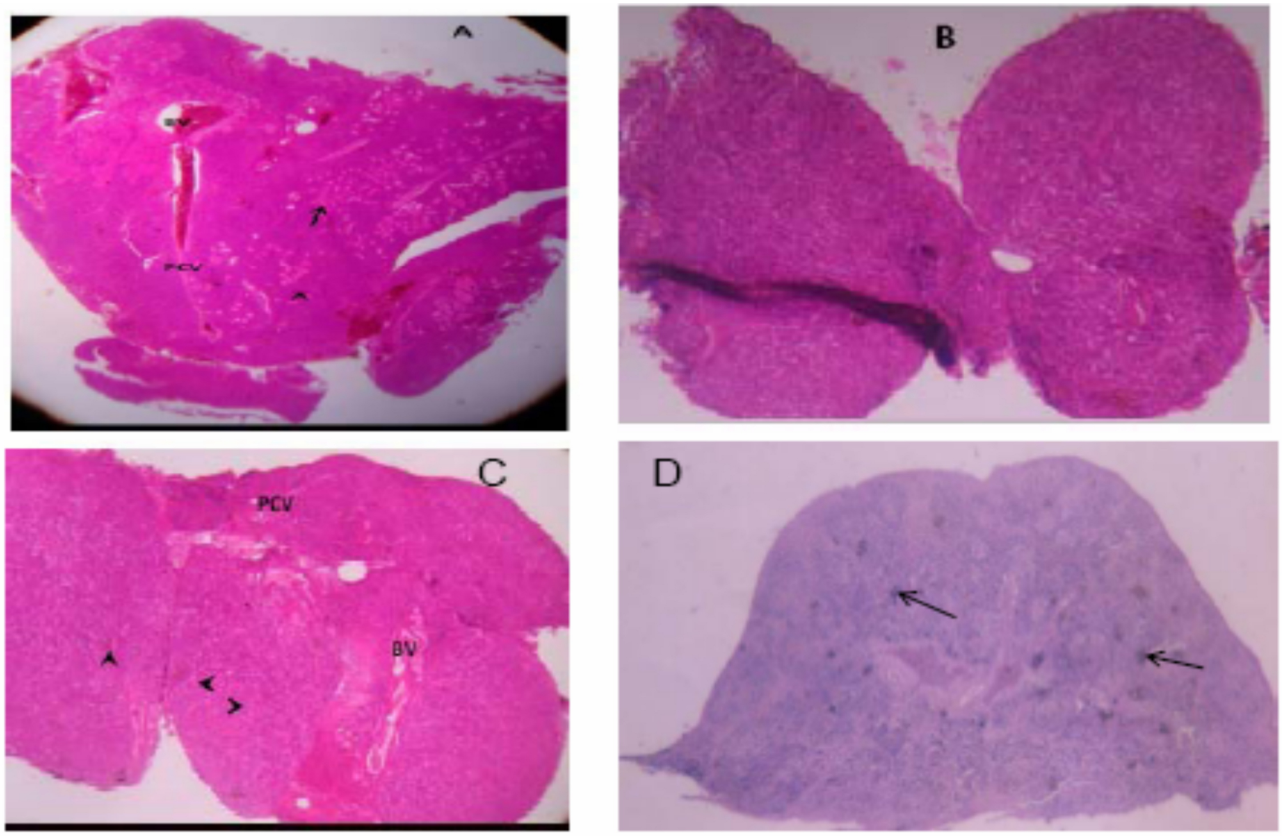

Fig. 3 : Light micrographs (100x) of head kidney of *M. vitttatus* during (A) Summer; (B) Pre monsoon; (C) Monsoon; (D) Post monsoon showing. Glomerulus, blood vessels (BV), MMCs (arrow) and posterior cardinal vein (PVC)

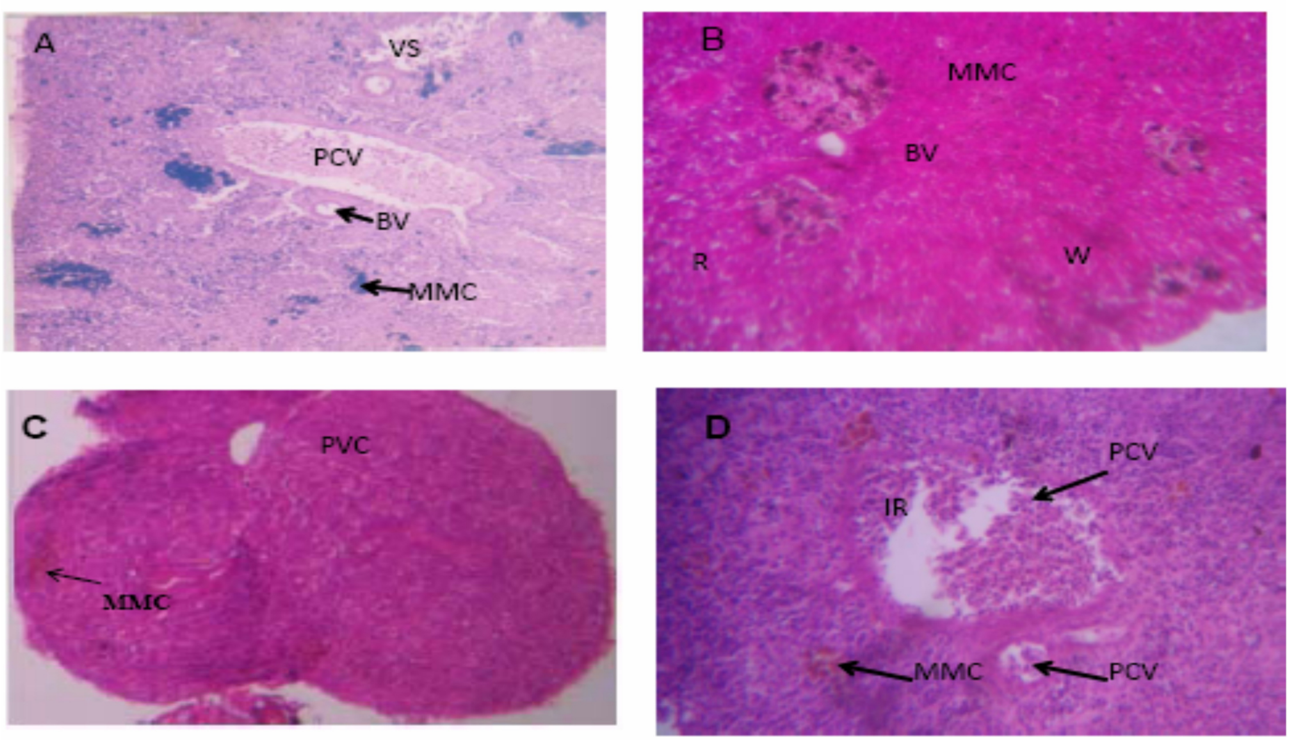

Fig. 4 : Light micrographs (A-D) of head kidney of *M. gulio* during (A) Summer; (B) Pre monsoon; (C) Monsoon; (D) Post monsoon. Section shows melanomacrophage centers (MMC),white blood cells (W), blood vessels (BV), interrenal gland (IR), venous sinus (VS) and post-cardinal vein (PCV)

Materials and Methods

Study area and experimental design: The Vellar estuary like other estuaries in tropical regions is characterized by a predominant monsoon (1000 mm) regime during October to December. During January–September, the climatic conditions are warmer and moderately warmer. Fishes such as *Mystus gulio* (25±10.5 g) and *Mystus vittatus* (30±10 g) were collected from the Vellar estuary during 2009-10 in the first week of May, August, November and January representing summer, premonsoon, monsoon and post monsoon seasons. The collected fishes were kept in fibre-reinforced plastic tanks with a 500 l water and the experiments were carried out immediately. During the sample collection, physico-chemical parameters of the water were analyzed (Sithik *et al.,* 2009). Blood samples were collected from healthy fishes. In order to sample the blood, fishes were quickly caught, placed in anesthetic (0.1 ml / 1-2-phenoxyethanol) and sampled through caudal puncture with a syringe. A part of fresh blood was placed in heparinized tube for nitroblue tetrazolium (NBT) test and white blood cells count (WBCs). The rest of the blood was left to clot at 4^{o}C for 2 hr, the clot was removed after centrifugation, and the serum aliquoted and stored at -20^{o}C for lysozyme activity. The immune organs like head, kidney and spleen were removed and placed into the tube contained bouin's fluid solution.

Total white blood cell counts: To determine total WBC, a 1 in100 dilution of the blood was made in phosphate buffered saline (PBS, 0.02 M, pH 7.3). Counts were carried out using a Neubauer haemocytometer (Hawksley and Son, England) and were expressed as cells ml^{-1}.

Serum lysozyme activity: Lysozyme activity was measured by the method of Ellis (1990) with minor modifications. In this turbidometric assay, 0.03% lyophilized *Micrococcus lysodeikticus* in 0.05 mM sodium phosphate buffer (pH 6.2) was used as substrate. Ten microlitre of fish serum was added to 250 µl of bacterial suspension in duplicate wells of a "U" bottom microtitre plate and the reduction in absorbance at 490 nm was determined after 0.5 and 4.5 min of incubation at 22^{o}C using a microtiter plate reader (Biorad, USA). One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 min^{-1}.

Nitroblue tetrazolium assay: The nitro blue tetrazolium (NBT) was measured by following the method of Munoz *et al*. (2000). Serum (100 µl) was placed in a microplate and incubated for 30 min at room temperature. The supernatant was discarded and 50 µl of 0.3% NBT were added and incubated for 2 hr at room temperature. The supernatant was again discarded, and the ELISA plates were fixed with 200 µl of absolute ethanol. Plate was washed twice with 200 µl of 70% methanol and allowed to dry. The formazan deposits generated were dissolved in 120 ml of 2M KOH with 140 ml of dimethyl sulfoxide (DMSO), and the activity was recorded at 620 nm using a microtiter plate reader.

Histology: The two immune organs i.e. head-kidney and spleen fixed in bouin's fluid for 24 hr, processed, embedded in paraffin wax were cut (WESWOX MT10908 Microtome) into 6 µm thick sections and stained in Haemotoxylin and Eosin (HE). The sections were observed under light microscope (Magnus MLX, India).

Statistical analysis: Mean and standard deviation (X ± SD) were calculated for each parameter. $P<0.05$ was taken to indicate a statistically significant difference.

Results and Discussion

Maximum salinity (37‰) was observed in water during summer season and minimum (4‰) monsoon due to mixing of fresh water through rain fall and precipitation. Salinity is the one of the important key factor in the marine environment. The fluctuations in salinity, affect the biological characteristics of the marine environment.

The maximum pH (8.0) was observed in summer period and minimum (6.5) during monsoon period. Maximum temperature (36.5^{o}C) was observed during summer season and minimum (27.0^{o}C) was observed in monsoon season. The spatial variation observed in temperature could be due to the variable intensity of prevailing currents and the consequent mixing of water (Reddi *et al*., 1993).

The non-specific immune system plays a major role in the immune response of teleost fishes because have only one type of immunoglobulin,IgM like. Adherence/ NBT (nitroblue tetrazolium) and respiratory burst process assay spot the light on the non-specific immune response and the antibacterial mechanisms of the tested substances (Ibrahem *et al*., 2010). A general seasonal variation was observed for WBCs, lysozyme activity, NBT activity and histology of the fish samples. The highest WBCs were observed in *Mystus gulio* at $10.3x10^{6}$ ml^{-1} during summer season and the lowest counts ($6.8x10^{6}$ ml^{-1}) were observed during monsoon season in *Mystus gulio* (Table 1). These results are in line with Morgan *et al*. (2008) who studied immune response of rainbow trout *Oncorhynchus mykiss* in commercial ponds. Collazos *et al.* (1998) reported that the leucocyte counts for both male and female tench (*Tinca tinca*) were significantly lower in spring and winter, when compared with summer and autumn, and this has since been corroborated by De Pedro *et al.* (2005). Many fish species showed lower blood lymphocyte

Table 1 : Non-specific immune parameters (WBC counts, lysozyme and NBT activity) of *Mystus* sp.

Seasons	No. of fish sampled per season		WBCs count ($x10^6$ ml^{-1})		Lysozyme (U min^{-1} ml^{-1})		NBT activity (OD at 620 nm)	
	Mystus gulio	*Mystus vittatus*	*Mystus gulio*	*Mystus vittatus*	*Mystus gulio*	*Mystus vittatus*	*Mystus gulio*	*Mystus vittatus*
May (summer)	40	35	10.3±0.56	10.1±0.46	1000±40	960±61	0.012±0.01	0.2±0.01
August(pre monsoon)	35	20	9.7±0.2	9.3±0.58	1180±62	1100±81	0.42±0.025	0.6±0.09
November (monsoon)	25	30	6.8±0.25	7.0±0.26	1400±89	1420±91	0.68±0.036	0.65±0.021
January (post monsoon)	45	40	8.8±0.36	8.0±0.39	1540±87	1525±110	0.53±0.042	0.55±0.026

counts and suppressed immune responsiveness to antigen in winter, even though the temperature is adequate for their survival (Wishkovsky and Avtalion, 1987).

Lysozyme is a fish defence element, which causes lysis of bacteria and activation of the complement system and phagocytes by acting as opsonin (Magnadottir, 2006). Among the sampling periods, the lysozyme activity was found at peak in post monsoon season in *M. gulio* (1540 U min^{-1} ml^{-1}) and lower in *Mystus vittatus* (960 U min^{-1} ml^{-1}) during the summer season (Table 1). Similarly, several earlier reports revealed that lysozyme level in *S. aurata* was less sensitive than *Clarias batrachus* which showed more sensitivity to seasonal or temperature changes (Hernandez and Tort, 2003; Kumari *et al.,* 2006). In contrast, Tort *et al.* (2004) reported that the lysozyme level decreased in *S. aurata* during low temperature and increased when the temperature increased.

The NBT assay is indicative of oxidative radical production from neutrophils and monocytes for use in defence against pathogens (Anderson and Siwicki, 1995). The maximum activity was observed at 620nm in *Mystus gulio* (0.68) during monsoon and lower (0.012) during summer season in the same species (Table 1). Earlier studies have also shown that the adaptation to lower temperature led to an increase in the respiratory burst activity in fish (Le Morvan *et al*., 1998; Kumari *et al*., 2006).

Histological observations showed differences with respect to seasons (Fig. 1, 2). The spleen in both fishes showed densely packed WBC and melanomacrophage centers (MMCs), during summer and pre-monsoon, whereas during monsoon and post monsoon normal WBCs and loosely packed MMCs were seen. Therefore, fish spleen plays a vital role in immune response and high production of melano-macrophages. This may be the reason that fish was affected by the environment.

Head kidney having the major part in immune response, also the WBCs and MMCs were found to be high during summer and pre-monsoon; whereas in monsoon it was adversely reduced. The teleostean kidney is one of the first organs to be affected by contaminants in the water (Thophon *et al.,* 2003).

Takashima and Hibiya, (1995) reported the most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman´s space.

In the present study, light micrographs of kidney of both the fish showed melanomacrophage centers,white blood cells, blood vessels, interrenal gland, venous sinus and post cardinal vein during different seasons. During summer season (Fig. 3, 4) results highly showed cloudy swelling in tubule cells, sinus and MMC in *Mystus gulio* and *Mystus vitatus* as compared to other seasons. Takashima and Hibiya (1995) stated that initial stage in the degeneration process can progress to hyaline degeneration, characterized by the presence of large eosinophilic granules inside the cells. The present study indicates that the kidney suffered damage during that season.

A number of reports have stated that the changes in non-specific immune parameters with relation to infection, toxicity, diet, stressors, pollution or temperature fluctuations in different species (Ellis, 2001).

Our results confirmed that the WBCs count of both catfishes were increased during summer season and decreased in monsoon. In contrast, lysozyme and NBT activity were decreased during summer season. During the monsoon season fishes were susceptible to infection due to reduced count of WBCs.

Acknowledgments

The authors are grateful to Dr. T. Balasubramanian, Dean, Faculty of Marine Sciences, Annamalai University for his support and encouragement and University Grants Commission, New Delhi for providing financial assistance.

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