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Research Article

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Seasonal variation in immune organs and immune response of catfish *Arius maculatus* in Parangipettai coastal area

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ABSTRACT

Different non-specific immune parameters and their seasonal changes in spotted Catfish Arius maculates from Vellar estuary was investigated. It was undertaken for a year which included four main seasons of a year such as summer (April-June), Pre Monsoon (July-september), Monsoon (October-December) and Post Monsoon (January-March). The Physico-chemical parameters (Temperature, pH, Salinity and Rainfall) of Vellar estuary and Non specific immune response (WBCs, Lysozyme and superoxides) and histological differences of the candidate species were observed during the different seasons. The results of physic-chemical parameters stated that the range was higher in summer and lower in Monsoon season except rainfall. White blood cells count (WBCs) and Serum lysozyme and myeloperoxidase activities were lower (6.2±0.1 and 960±61, respectively) in Monsoon as co mpared to any other season of the year. The NBT activity was higher (0.65±0.021) in Monsoon season and lower in summer. In Histology, the Melano Macrophage Centers and WBCs were densely packed in summed and loosely packed in Monsoon season. This study indicated that certain non-specific immune parameters of this species can be modulated at certain times of the year.

Key words: *Arius maculatus*, Non specific immune response, seasonal variation, Histopathology, Vellar estuary.

INTRODUCTION

The environment is a generic term to describe all living and non-living things that occur on the planet or a part of it. Within this, a greater nutrient load in an aquatic environment which results in more life that can be supported, the aquatic environment is more stable, aquatic organisms are seldom exposed to desiccation, oxygen and light can become limiting factors in an aquatic environment, and aquatic organisms are less influenced by gravity. The factors that will be considered include; photoperiod, temperature, pH, oxygen level, particulates, and salinity. Among the different environmental factors, temperature is very critical which can disturb the homeostasis in a living animal [1]. The immune system of a poikilothermic vertebrate like fish is affected by seasonal temperature [2-7]. In many fishes, reduced immunity is often recorded during cold periods due to the low temperature [8-10]. The immune system of the teleost fish contains various mechanisms of non-specific (innate or natural) immunity and specific (acquired or adaptive) immunity that determine resistance against pathogenic or parasitic organisms [11]. 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 (MPO), superoxides, lysozyme, complement, acute phase proteins, interferons, agglutinins, properdins and lysins] contribute to the general immunological defence mechanism [12].

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 [13]. Also, the estuaries continually receive anthropogenic inputs and contain many chemicals that are potentially toxic to aquatic organisms [14-15]. The Vellar estuary like other estuaries in tropical regions is characterized by a predominant monsoon (rainfall ca. 1000 mm) regime, for during October to December. During January–September, the climatic conditions are warmer and moderately warmer.

Spotted catfish, Arius maculatus (Thunberg, 1792), is a benthic species in tropical and subtropical waters, inhabiting the bottom of estuaries, rivers and coasts. Several workers have studied the biology of cat fishes from Indian waters [16-17]. The aim of the present study was to investigate some of the non-specific immune parameters and their seasonal variations using the edible fishes *Arius maculates* as representative species.

EXPERIMENTAL SECTION

Fish

Catfish *Arius maculates* (31±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 (FRP) tanks with a 500 L water volume each and the experiments were carried out immediately.

Blood collection

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 and gill 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^oC for 2 h, the clot was removed after centrifugation, and the serum aliquoted and stored at -20^oC for lysozyme activity. The immune organs head kidney and spleen were removed and used for histological analysis through cryo section.

Water quality analysis

During the sample collection, physico-chemical parameters such as temperature, Salinity, pH and rainfall of the water were measured [18].

Assays

Total white blood cells count (WBCs)

To determine the total white blood cells count (WBCs), a 1 in100 dilution of the blood was made in phosphate saline buffer (PBS, 0.02 M, pH 7.3). Counts were carried out using a Neubauer heamocytomater (Hawksley &Son, England) and were expressed as cells ml^{-1} [19].

Lysozyme activity

Lysozyme activity was measured by the method of Ellis [20] 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^oC using a microtiter plate reader (Biorad, USA). One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 per min.

NBT assay

Respiratory burst activity of blood sample was quantified by the nitroblue tetrazolium (NBT) assay [21], which measures the quantity of intracellular oxidative free radicals. This method was slightly modified by changing the concentration of NBT solution to 0.2%.

Histological study

Cryostat sections, 5 mm in thickness (model; Leica 1850, Germany), of the immune organs spleen and head kidney were performed for light microscopic study and the thin sections were stained with hematoxylin and eosin stains and observed through light microscope [22].

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

The levels of different water quality parameters of Vellar estuarine waters are presented in Table 1. The levels of different blood non-specific immune parameters such as white blood cells (WBCs), Lysozyme and superoxide anions are given in Table 2. The WBCs count was higher during the period of summer (10.3±0.23) and lower in counts (6.2±0.1) were observed in monsoon season. The lysozyme level was higher in the period of summer (1225±91) and lower during winter (960±61). The respiratory burst activity was reached peak (0.65±0.021) during monsoon and lowest in the period of summer season (0.24±0.01). The significant difference values (P) in all the three assays were < 0.05. Histological observations showed differences with respect to seasons (Figs. 1 and 2). The results of spleen showed densely packed white blood cells (WBC) and melanomacrophage centers (MMCs), during summer and pre-monsoon, whereas during monsoon and post monsoon normal WBCs and loosely packed MMCs were seen. The WBCs and MMCs were found to be higher during summer and pre-monsoon; whereas in monsoon it was adversely reduced. In the present study, kidney section shows Melanomacrophage centers (MMC), White blood cells (W), Blood vessels (BV), venous sinus (VS) and Post cardinal vein (PCV) during different seasons. During summer season, the results highly showed cloudy swelling in tubule cells, sinus and MMC in all the three species compare to other seasons.

DISCUSSION

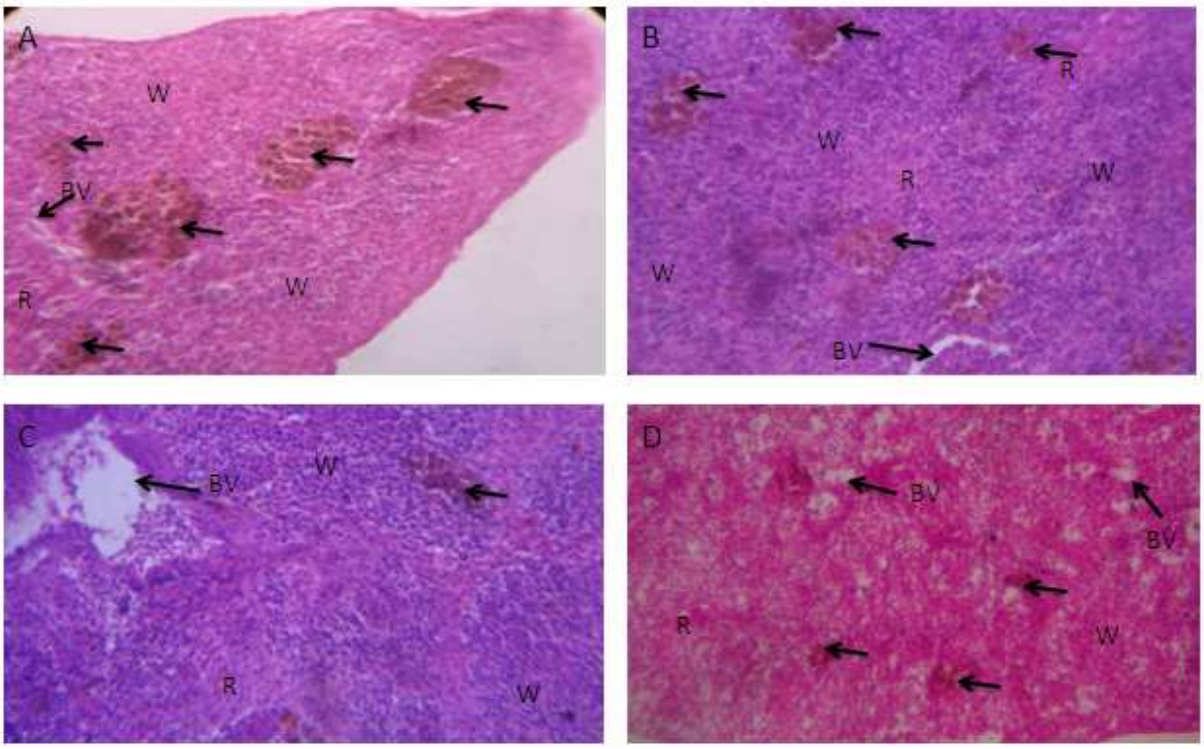
Seasonal cycles can affect several biological activities such as behaviour, feeding, metabolism, immunity and reproduction in fishes [23-26]. Fish immunity is affected by many parameters in the environment; water temperature being considered as the leading factor. However, the experimental study showed that seasonal effect is a stronger factor than water temperature [27]. In our study the water temperature range was between 27-36.5°C. The Centropomidae when exposed to temperatures below 14 °C, which may reduce feeding, lose equilibrium and die [28-29]. Therefore, the temperature fluctuation occurrence is not a major problem to the fishes in vellar estuary. In fish, the blood levels of biochemical variables have turned out to be mainly affected by photoperiod, water temperature, dissolved oxygen, salinity, quality and rate of food consumed [30-33]. In our study the WBCs were higher in summer season and lower in monsoon season. These results are in line with Morgan *et al*. [19] who studied immune response of rainbow trout *Oncorhynchus mykiss* L. in commercial ponds. Collazos *et al.* [34] reported that the leucocyte counts for both male and female tench *Tinca tinca* L. and were significantly lower in spring and winter, when compared with summer and autumn, and this has since been corroborated by De Pedro *et al.* [35]. The circulation levels of different blood cells like erythrocyte count, leucocytes count and percentage of haematocrit values also varies seasonally [36]. The nonspecific immune response of fish includes cellular (phagocytes) and humoral components (complement system mainly) or the systemic inflammation. In several reports, lower values of water temperature cause the suppression of acquired immune system measured for instance by lymphocyte activity and antibody production, but innate components of the immune system are relatively independent of temperature [37].

Lysozyme is one of the important bactericidal enzymes of innate immunity. It is involved in the overall alarm responses during the infection as well as stress conditions [38] and acts as an acute phase protein [39]. Its functional role in fighting against infectious diseases of fish has already been recorded [440-43]. In our study, the lysozyme level was peak in summer and lowest in monsoon season but there is not many significant differences occurred. One study revealed that lysozyme level in *S. aurata* was less sensitive to seasonal or temperature changes [44]; whereas a clear reduction in lysozyme level was marked in *S. aurata* at lower temperature [45]. But It was consequently suggested that temperature had a limited affect on lysozyme activity in the Nile tilapia [46].

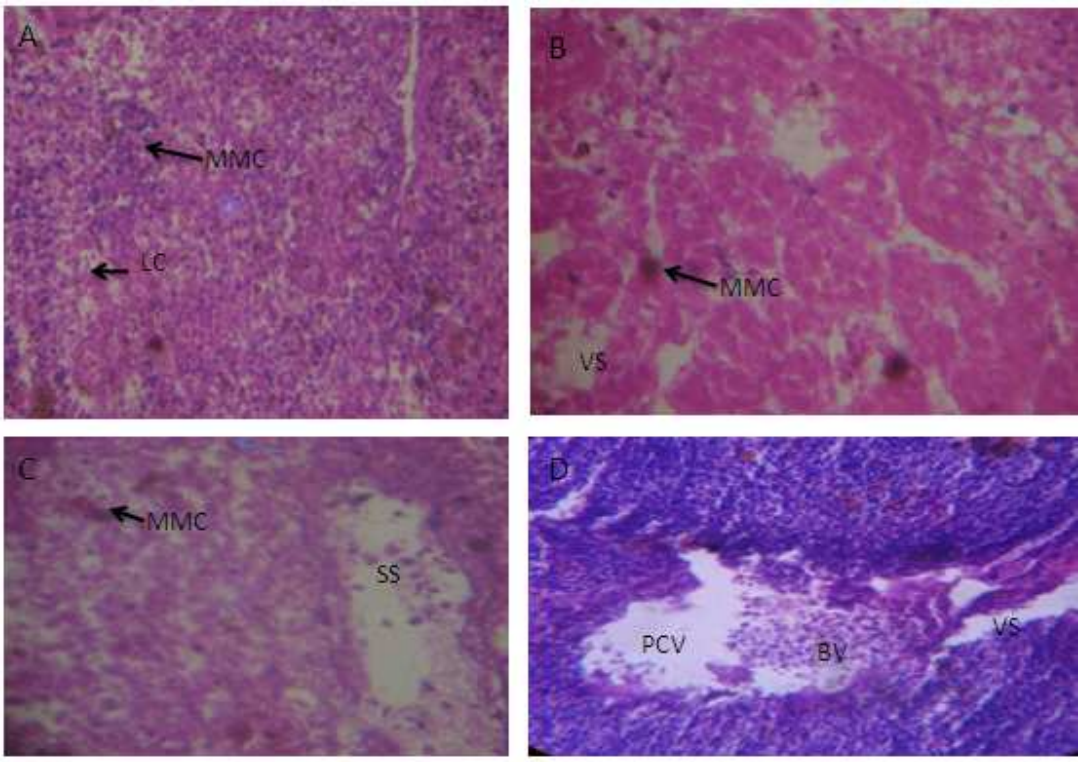
Phagocytes produce toxic oxygen forms during a process called respiratory burst [47]. Since superoxide anion is the first product to be released from the respiratory burst, the measurement of O_2^- has been accepted as a precise way of measuring respiratory burst [48]. The nitroblue tetrazolium (NBT) assay is indicative of oxidative radical production from neutrophils and monocytes for use in defence against pathogens [49]. In this present study the maximum activity was observed during monsoon and lower during summer season in the candidate species. Earlier studies

have also shown that the adaptation to lower temperature did lead to an increase in the respiratory burst activity in fish [50-53]. In general, parameters are suppressed during winter and raised in summer [54].

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory [55-56] and field studies [57-59]. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish [60]. In our study the cells were densely packed in warmer conditions and loosely packed in cold conditions. Furthermore, the alterations found in these organs are normally easier to identify than functional ones [61], and serve as warning signs of damage to animal health [62]. The teleost kidney is one of the first organs to be affected by contaminants in the water [58]. 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 [63]. The head of kidney containes endocrine elements, the chromaffin cells and interrenal tissue which are located around the blood vessels. Our results confirmed that the WBCs count and lysozyme activities of catfish were increased during summer season and decreased in monsoon. In contrast, NBT activity was decreased during summer season. Therefore, the activities of immune molecules were produced highly from immune cells during monsoon season to combat invading pathogens. Moreover, during the monsoon season fishes were susceptible to infection due to reduced count of WBCs.



Light micrographs of Head Kidney in *Arius maculatus* during different seasons ((A-summer, B-premonsoon, C-monsoon, and D-post monsoon). Section shows Melanomacrophage centers (MMC), Lymphocyte chord (LC), venous sinus(VS), Blood vessels (BV) and posterior cardinal vein (PCV) . H&E . (600 X).



Light micrographs of Head Kidney in *Arius maculatus* during different seasons ((A-summer, B-premonsoon, C-monsoon, and D-post monsoon). Section shows Melanomacrophage centers (MMC), Lymphocyte chord (LC), venous sinus(VS), Blood vessels (BV) and posterior cardinal vein (PCV) . H&E . (600 X).

Table 1

Seasons	Salinity (ppt)	pH	Temperature (°C)		Rainfall (mm)
May	37	8.0	36.5	27.5	0020
August	30	7.4	34.5	25.8	0230
November	04	6.7	30.0	24.0	8240
January	28	7.1	33.0	28.0	0079

Table 2

Seasons	WBCs	Lysozyme activity	NBT assay
Summer	10.3±0.23	1225±91	0.24±0.01
Pre Monsoon	9.9±0.47	1100±81	0.37±0.07
Monsoon	6.2±0.1	960±61	0.65±0.021
Post Monsoon	9.1±0.41	1115±110	0.55±0.026

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