Effect of seasonal variations in haematology, biochemical, immunity constituents in *Mugil cephalus, Chano chanos,* and *Arius arius* on Southeast Coast of Tamil Nadu, India

Abstract

The assessment of the effect of seasonal variations through health, haematology and serum biochemical components of three marine teleost fishes, *Mugil cephalus*, *Chanos chanos*, and *Arius arius* from Kovalam, East Coast Road, Southeast coast of Tamil Nadu, India. The studies pointed out the seasonal variation of fish health and immunity. During summer and pre-monsoon, a total number of 20 samples were collected from each sample, and haematological, biochemical, immunity parameters and fish health were considered season-wise. The weight and length were calculated. The WBC, RBC, Hb, and HCT were higher during summer in *M.cephalus*, *C.chanos*, and *A.arius* likewise these parameters were lower in the pre-monsoon period ($P<0.05$). The MCV, MCH and MCHC, and ESR of all three species were higher in pre-monsoon whereas in summer it decreased. Likewise, the leukocytes such as neutrophils, monocytes, lymphocytes, eosinophils and thrombocytes show minimum and pre-monsoon, attain maximum levels in the summer season. Most importantly the serum components protein and glucose ($P<0.05$) were higher in summer whereas cholesterol and urea were lower and vice-versa. Although IgM and IgG level was slightly higher in summer and declined in pre-monsoon. Lysozyme assay, respiratory burst activity (NBT) reveals a higher concentration in pre-monsoon and was lower in summer. Based on the findings the report indicates the seasons mainly impact the physiological status of the fish species.

Keywords: *M.cephalus*, *C.chanos*, *A.arius*, seasonal, haematology, biochemical, immunity

Introduction

A very long ago, fish consider protein-rich, easily digestible, and have many therapeutic properties such as muscular degeneration, heart diseases, mental health and so on (Begum *et al*., 2013) [4]. Forthcoming studies of the fish state the components of the body varied with season, age, feeding behaviour, sex, and environment (Norouzi & Bagheri, 2015) [20]. Fish emulate the condition of water contamination and quality even now it exists at a low level in the food chain of an aquatic ecosystem. They can acquire and hold heavy metals, pesticides and chemicals utilizing a compliant manner hence the contamination in their circumstances was deducted. Fish intake an enormous amount of various aquatic feeds along with infected compounds which were subsequently bioaccumulating the chemicals in the organs and body tissues of the fish. Water is consumed by a human for waste expulsion and decontamination. Water quality is affected by biological and physical processes that influence the cause of water contamination prompting the composition of aquatic and health sources (Ambreen and Javed, 2018; Tahir *et al.,* 2021) [2, 27].

Understanding the effects of the season fish quality, and composition can provide to select good fish. The fish health assessment helps evaluate the fish's haematology (Fazio et al., 2016) [7]. Various studies stated the impact of environmental changes influence on the haematology of the fish. The condition of the immune system at low

temperatures is suppressed in the state. As the result, the blood count and other main physiological parameters may vary with the seasonal variations (Habib *et al*., 2021) [13].

Haematology and serum analysis plays a vital role in the pathophysiological of fish. It is an indicator of stress and infections which are caused by pollution. The blood cell count shows the body conditions before the disease was identified and the infection affected (Ali and Rani, 2009) [1]. Fish skin, gills and alimentary tract absorb contaminants and diffuse them into various tissues and organs, affecting their physiological functions (Tahir *et al*., 2021) [27]. In the gills, oxygen consumption is prominent and these are more affecting organs due to contaminants, which may alter biological and physicochemical properties of variation. The effluents, heavy metals, and pesticides are causing disease and may thread to aquatic forms (Panigrahi *et al*., 2014; Yadav *et al*., 2018a) [21, 28]. The necessary parameters are haematology and biochemical, and immunological exhibit the health status before and after the fish was stocked. Because seasonal stress conditions, food scarcity, and other environmental statuses affect the fish direct or indirectly. The cell count such as RBC, WBC, ESR, and HCT varied in certain factors in water (Ghaffer *et al*., 2019, 2018) [9, 10].

The fundamental and for most immunity is an innate immune system that acts as a defence aspect. The lysozyme activates and destroys the invading pathogens and eliminates the invaders from the body of fish. The NBT (Nitroblue tetrazolium) assay exhibits the production of oxidative radicals from monocytes and neutrophils in the defence mechanism (Balamurugan *et al*., 2012) [3]. The present study exhibit to present to initiates a few facts about the health conditions of economically valuable fish species *M.cephalus*, *C.chanos*, and *A.arius* and it helps to enhance the production in summer and pre-monsoon seasons. In elaborate view that haematology, biochemical and immunity play a vital role in indicating species physiology.

Material and Methods

Blood collection and preservation

During summer and pre-monsoon, blood samples were collected from several 20 species for each sample, size ranging between 120 to 175 g and 18 to 25 cm in the Kovalam, East Coast Road, Southeast coast of Tamil Nadu, India. The blood was collected from an anaesthetized fish caudal vein very gently using a disposable heparinized syringe. Eventually, the blood was stored in Eppendorf vials coated with EDTA anticoagulant. For serum separation, the blood was collected in clotting tubes without EDTA. The blood was clotted and the serum was separated by 350 rpm centrifugation.

Determination of haematological Parameters

To determine the blood cells, firstly the caudal fin was sterilized using a 70% alcohol swab to avoid contamination the caudal vein was pricked with a needle. The blood was collected from the caudal vein very gently using a disposable heparinized syringe. Eventually, the blood was stored in Eppendorf vials coated with EDTA anticoagulant. The improved Neubauer's haemocytometer was used to determine the Red Blood Cells (RBC) and White Blood Cells (WBC). The Packed Cell Volume (PCV) and Erythrocyte Sedimentation Rate (ESR) were

estimated by earlier protocols (Faheem *et al*., 2021). Anticoagulated blood was kept in the test tubes for one hour without disturbed. The erythrocytes were settled in the bottom and it was measured in mm/h. Haemoglobin (Hb) is estimated by a Sahli's haemoglobinometer. MCV, MCH, and MCHC were calculated by average values of Hb% and Haematocrit (HCT) for the micro-haematocrit method was calculated using Dacie and Lewis (1977) [5]. For leukocyte count, a blood smear was prepared using Wright-Giemsa stain (Ghaffar *et al*., 2017) [11] and the smear was examined by light microscope under 100X magnification.

Determination of serum and immunological constituents

The biochemical parameters glucose and protein estimation was followed by standard protocol suggested by the Phosphomolybdate method and Lowry method respectively (Nimmy and Pawlin, 2018) [19]. The cholesterol and urea were determined using the standard method suggested by Francesco *et al*, (2012) [8]. The determination of immunoglobulin IgM and IgG ELISA kit (EMP, M201, Microplate Reader) assay procedure was followed (Nimmy and Pawlin 2018) [19].

Lysozyme assay

The lysozyme assay method was followed by previous methods (Sakthivel *et al*., 2012) [24]. It was a turbidometric assay for substrate 0.03% lyophilized Micococcus lysodeikticus was added into 0.05mm sodium phosphate buffer pH value 5.9. Then 10µl of fish serum mixed with 250µl of bacterial suspension in U bottom duplicate well plate in microtiter (EMP, M201, Microplate Reader, USA) and reduction in absorbance were determined at 520nm in 5min o incubation at 24^0C (LABINDIA analytical, UV 3200, UV-Vis Spectrophotometer). 1U of lysozyme was measured at 0.001 per min of absorbance.

Respiratory burst activity

For respiratory burst activity, the assay procedure was followed by (Balamurugan *et al*., 2012) [3] the reduction of NBT (nitroblue tetrazolium) measured in formazan reactive oxygen radical. 40µl of blood was taken in a microtiter (EMP, M201, Microplate Reader, USA) and incubated in a 1hr water bath and then washed with 90µl PBS to clear non-adherence cells. Add 0.2% of NBT (100µl), and incubate the solution at room temperature. 100% methanol ((100µl) was used as a fixative solution, after that 70% methanol was used to wash. 2N KOH with 140µl DMSO. The mixture was shaken well and take absorbance at 630nm (EMP, M201, Microplate Reader, USA).

Statistical assay

All values are illustrated as the Mean ± Standard error of the mean. Results were evaluated normally and distributed with normality. One-way variance (ANOVA) followed Turkey's test was used for analysis. Statistically significant P values (<0.05) are considered.

Results

Effect of seasons in haematological parameters

In this report, haematology was investigated through White Blood Corpuscles (WBC), Red Blood Corpuscles (RBC), Haemoglobin (Hb), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), differential count thrombocytes, neutrophils, lymphocytes, eosinophils and monocytes were examined and detailed results are interpreted in tables 1&2. The values of RBC, WBC, Hb, and HCT are maximum in summer and MCV, MCH, and MCHC were minimum in the summer. Whereas MCV, MCH, and MCHC are higher and RBC, WBC, Hb, and HCT were lower significantly ($P<0.05$) in the pre-monsoon season (Tables 1&2) in all the three *M.cephalus*, *C.chanos,* and *A.arius* species. The highest leukocytes such as thrombocytes, neutrophils, lymphocytes and eosinophils, and the lowest in pre-monsoon (Tables 1&2). While in between these three fishes *M.cephalus* values are maximum than the other two *C.chanos* and *A.arius* species in both seasons.

Table 1. Seasonal variations in haematological parameters of *M.cephalus*, *C.chanos*, *A.arius*, in summer

Haematology	***M.cephalus***	***C.chanos***	***A.arius***
T.length (cm)	23.86±1.03	22.36±1.17	22.62±1.08
T.weight (g)	171.22±35.42	163.03±33.32	168.72±35.04
WBC ($\times 10^6$ µl)	28.09±1.04	26.43±1.48	28.30±1.57
RBC ($\times 10^3$ µl)	2.56±0.25	2.62±0.53	2.01±0.08
Hb (%)	39.04±1.39	36.82±1.68	36.05±1.43
HCT (%)	28.17±0.13	27.08±0.11	26.72±0.16
MCV (fL)	17.05±1.04	16.75±1.51	16.49±1.17
MCH (pg)	149.08±3.81	182.36±10.06	173.14±3.22
MCHC (%)	130.62±6.63	129.51±13.01	126.09±12.27
ESR (mm/h)	0.48±0.25	0.52±0.18	0.45±0.27
Thrombocytes (%)	30.08±4.62	29.13±5.01	30.45±5.38
Monocytes (%)	10.68±1.06	10.24±1.17	9.92±1.35
Lymphocytes (%)	81.01±2.15	80.76±2.07	80.52±2.19
Neutrophils (%)	12.22±1.60	12.08±1.19	12.03±1.63
Eosinophils (%)	1.08±0.33	1.09±0.18	1.08±0.41

Table 2. Seasonal variations in haematological parameters of *M.cephalus*, *C.chanos*, *A.arius*, in pre-monsoon

Haematology	***M.cephalus***	***C.chanos***	***A.arius***
T.length (cm)	22.13±1.04	21.61±1.27	20.76±1.38
T.weight (g)	169.19±32.15	162.15±32.13	166.42±31.02
WBC ($\times 10^6$ µl)	26.62±1.08	25.32±1.09	27.12±1.25

RBC ($\times 10^3$ µl)	2.18±0.05	2.54±0.29	1.87±0.02
Hb (%)	38.48±1.49	35.62±1.38	35.05±1.03
HCT (%)	27.43±0.25	26.03±0.07	25.62±0.53
MCV (fL)	17.97±1.09	17.05±1.03	17.42±1.54
MCH (pg)	150.17±3.22	183.13±9.43	174.68±3.21
MCHC (%)	131.21±5.41	130.04±12.37	127.76±13.66
ESR (mm/h)	1.02±0.43	1.16±0.28	1.05±0.08
Thrombocytes (%)	29.94±4.03	28.60±4.43	29.45±4.67
Monocytes (%)	10.02±1.06	9.12±1.03	9.18±1.02
Lymphocytes (%)	79.57±1.05	78.41±1.33	79.84±2.03
Neutrophils (%)	11.87±1.04	11.69±1.08	11.92±1.16
Eosinophils (%)	0.95±0.09	1.01±0.03	0.89±0.18

Effect of seasons on biochemical and immunological parameters

In biochemical parameters the serum glucose, protein, cholesterol and urea contents are estimated seasonally. The glucose and protein were recorded as an increase in summer in all three species likewise cholesterol and urea decreased in summer. Hence in pre-monsoon, the highest values of cholesterol and urea and the lowest values of protein and glucose were recorded (Table 3&4).

Table 3. Seasonal variations in serum biochemical & immunological parameters of *M.cephalus*, *C.chanos*, *A.arius*, in summer

Biochemical	***M.cephalus***	***C.chanos***	***A.arius***
Glucose (mg/dl)	88.04±1.68	85.18±1.53	85.13±1.72
Protein (mg/dl)	5.82±0.47	5.29±0.45	5.36±0.38
Cholesterol (mg/dl)	189.04±2.42	182.17±0.08	185.05±2.32
Urea (mg/dl)	6.62±0.16	6.31±0.08	5.83±0.12
Immunological			
IgM	8.53±1.08	7.92±1.32	7.50±1.62
IgG	7.77±1.23	7.82±1.03	7.49±1.14

Table 4. Seasonal variations in serum biochemical & immunological parameters of *M.cephalus*, *C.chanos*, *A.arius*, in pre-monsoon

Biochemical	***M.cephalus***	***C.chanos***	***A.arius***

Glucose (mg/dl)	85.73±1.15	82.49±1.02	82.63±1.38
Protein (mg/dl)	4.08±0.33	4.16±0.42	4.29±0.26
Cholesterol (mg/dl)	191.01±2.04	188.08±0.17	189.43±2.02
Urea (mg/dl)	6.85±0.16	7.08±0.05	6.12±0.09
Immunological			
IgM	7.84±1.02	7.01±1.05	6.95±1.06
IgG	6.53±1.21	6.82±0.83	6.24±1.02

Furthermore, in immunoglobulin, the IgM and IgG were significantly maximum in summer and it was minimum in pre-monsoon (Tables 3&4). For immunity, total leukocyte count, the lysozyme assay, and respiratory burst activity were noted as moderately highest in pre-monsoon and lowest in summer. All data were illustrated in tables (Table 5).

Table 5. The level of WBCs & Lysozyme activity & NBT activity in summer and pre-monsoon

Summer	***M.cephalus***	***C.chanos***	***A.arius***
T.leukocytes	28.09±1.04	26.43±1.48	28.30±1.57
Lysozyme activity	1218±89	1213±91	1208±77
NBT activity	0.25±0.01	0.26±0.07	0.24±0.21
Pre-monsoon			
T.leukocytes	26.62±1.08	25.32±1.09	27.12±1.25
Lysozyme activity	1253±84	1249±94	1262±80
NBT activity	0.76±0.04	0.68±0.07	0.72±0.18

Discussion

All over the world seasonal changes caused a significant impact on most species and the effect of seasonal fluctuation is well defined in haematological variation in fishes. The present observation reveals the seasonal changes and effects on haematological parameters of *M.cephales*, *C.chanos* and *A.arius*. The study of haematology has sensitive and effective parameters in the fish and has a higher significance to evaluate the pathological, and physiological alteration due to pollution in an aquatic ecosystem. Therefore, haematology is a big tool for identifying the fish's health status (Ali and Rani, 2009) [1]. Pesticides cause huge and fast alterations in blood cells (Tahir *et al*., 2021) [27]. Hence the haematological indices are efficient to obtain retort and health status in contamination exhibits in the aquatic environment (Pimpao *et al*., 2007) [22]. The seasons, gender, mass, propagation, health, water temperature, environment, nutrition and stress may be affected through haematological indices

(Hrubec *et al*., 2008) [14]. The haematological parameters like RBCs, WBCs, Hb, and HCT were high in the summer season while lower in pre-monsoon in all three species. It was because temperature, metabolic rate, and breeding also increase haematology was observed by Faheem *et al*, (2021) [6] in *L.rohita.* The concentration of Hb, HCT, ESR, thrombocytes and neutrophils were higher significantly in summer while these declined in pre-monsoon. Similar findings were noted in *M.armatus* (Narejo *et al*., 2002) [18]. In pre-monsoon, non-significant parameters are seen in haematology. More deviations were observed in selected species in summer as well as pre-monsoon (Gupta and Mishra, 2012) [12]. A review of blood counts and biochemical parameters in three species of Cauvery River was incorporated into our report (Mohan and Dhanapalan, 2015) [17]. The high ratio of RBCs and WBCs in this report and the inverse relation between the three fishes, such variation was due to the adaptation of fishes to their environmental conditions, the fish health affects subsequently (Faheem *et al*., 2021) [6].

In serum biochemical parameters mainly exhibits fish maturity, and monitor significant changes in fish health and water quality related to the ecosystem. Serum biochemical differences varied significantly in each species and may be influenced by seasons, temperature, food habits, sex, and age of the species (Jawad et al., 2004) [15]. The serum glucose and protein were in an increased state in summer whereas cholesterol and urea declined. This may probably be because of glycogen storage in the liver. Our findings are closely related to researchers who studied *L.calcarifer, M.cephalus*, and *C.chanos* (Satheeshkumar *et a*l., 2011) [25]. Moreover, cholesterol and urea values are increased during pre-monsoon while the protein and glucose were decreased condition. These levels of cholesterol indications due to lipid metabolism and liver dysfunction were closely related to previous reports (Kavadias *et al*., 2004) [16]. Haematological and biochemical parameters are tools for monitoring fish health in all seasons. The main aim to study the seasonal variations in fish is to determine the seasonal fluctuation of the haematological parameters. Different aspects of fish depended on the temperature in various seasons (Shahjahan et al., 2018) [26]. The main factor of immunity is water temperature. Water temperature controls the immunity of the fish. The immune pattern of *M.cephalus, C.chanos*, and *A.arius* the IgM and IgG levels higher in summer which was lower in pre-monsoon. During summer the immunoglobulins were higher due to the spawning season for selected fish. Meanwhile, pre-monsoon immunity factors are not necessary to relate to water temperature was corporates with seasonal changes in immune activities in *C.carpio* (Saha *et al*., 2003) [23]. The IgM and IgG imply a greater significance in the immune system of teleost fish.

The bactericidal enzyme of innate immunity is lysozyme. During stress conditions and infections, it overwhelmed responded in the acute phase protein act as a functional aspect against contagious disease. Here the lysozyme was moderately highest in the summer and declined in pre-monsoon. So, our report stated the lysozyme level in fish does not affect that much temperature and season. The NBT assay indicates monocytes and neutrophils are defensive against invaders in oxidative radical production. The toxic oxygen is produced by a phagocytic activity known as respiratory burst. In this study, the observed activities were during summer and pre-monsoon, in this season maximum activity was noted in summer and some were minimum during summer.

Conclusion

In conclusion, the results reported that seasons have a prominent effect on selected species. So, the seasons were taken to monitor and consider the health and immunity status of the selected fishes. Likewise, fish blood parameters also are considered to interpret fish health. For further studies our report suggested to the seasons are taken into consideration to monitor water changes.

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