Effect of seasonal variations in hematology, 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 on health, hematology, 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 objectives of the study pointed out the effect of seasonal variation on fish health and immunity. During summer and pre-monsoon, a total of 20 samples were collected from each sample, and hematological, biochemical, immunity parameters, and fish health were considered. *M.cephalus* (L-23.86±1.03; W-171.22±35.42) *C.chanos*, (L-22.36±1.17; W-163.03±33.32) and *A.arius* (L-22.62±1.08; W-168.72±35.04) have different length and weight. 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, MCHC, and ESR of all three species were higher in the pre-monsoon whereas in the summer they decreased. Likewise, leukocytes such as neutrophils, monocytes, lymphocytes, eosinophils, and thrombocytes show minimum levels in the pre-monsoon and attain maximum levels in the summer. Most importantly, in the summer, serum components protein and glucose were higher, while cholesterol and urea were lower, and vice versa. Although IgM and IgG levels (P<0.05) were higher in the summer and declined in the pre-monsoon season. In the lysozyme assay, respiratory burst activity (NBT) (P<0.05) reveals a higher concentration in the pre-monsoon and was lower in the summer. A total of thirteen hematological parameters, six biochemical, and two immunological parameters, and lysozyme and NBT activities showed a significant relationship with fish health throughout the seasonal changes. Based on the findings, *M.cephalus* shows maximum variations in length, weight, hematology, biochemical, and immunity levels, whereas *C.chanos* showed minimum variations. Hence the report indicates the seasons mainly impact the physiological status of the fish species.

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

Introduction

A very long time ago, fish consider protein-rich, easily digestible, and had many therapeutic properties such as muscular degeneration, heart diseases, mental health issues, and so on (Begum *et al*., 2013) [5]. Forthcoming studies on the state of the fish indicate the components of the body difference with the season, age, feeding behavior, sex, and environment (Norouzi & Bagheri, 2015) [27]. 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 in 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 subsequently bioaccumulate the chemicals in the organs and body tissues of the fish. A human being depends on water 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, 36].

Understanding the effects of the season on fish quality and composition can provide a basis for selecting good fish. The fish health assessment helps evaluate the fish's hematology (Fazio et al., 2016) [11]. Various studies stated the impact of environmental changes influence on the hematology of the fish. The condition of the immune system at low temperatures is suppressed in the state. As a result, the blood count and other main physiological parameters may vary with the seasonal variations (Habib *et al*., 2021) [20]. The study of hematology has been a diagnostic tool for disease and damage to the blood. It's an efficient one and gives clear insight into the health index and physiological and pathological status of different fish species. Hematology indicates the internal and external features of the environment. Continuous evaluation of blood includes various components such as RBC, WBC, Hb, Hct, etc., which are considered the main tools of diagnosis (Ahmed *et al*., 2020) [3].

Hematology and serum analysis plays a vital role in the pathophysiology of fish. It is an indicator of stress and infections that are caused by pollution. The blood cell count shows the body condition 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) [36]. In the gills, oxygen consumption is prominent, and these are more affected organs due to contaminants, which may alter the biological and physicochemical properties. The effluents, heavy metals, and pesticides are causing disease and may affect aquatic forms (Panigrahi *et al*., 2014; Yadav *et al*., 2018a) [28, 37]. Hematology, biochemical, and immunological parameters are required to demonstrate the health status before and after the fish stocking. Because seasonal stress conditions, food scarcity, and other environmental statuses affect the fish directly or indirectly. The cell counts such as RBC, WBC, ESR, and HCT varied in certain factors in water (Ghaffer *et al*., 2019, 2018) [16, 17]. More studies confirmed the blood and biochemical parameters of various fish species (Çelik 2005, 2004; Rudneva *et al*., 2010, 2009) [7, 8, 30, 31]. The fish were collected from the Southeast coast, and a detailed study of hematology, biochemical, and immunological parameters was analyzed. In particular, this study provides a reference statement for health and immunity indications. The measured values directly influence the seasons in the commercial areas (Ferri *et al*., 2022) [13]. The number of reports carried out to provide the ecological changes and blood parameters of the species. The immunity level subsided due to the low temperature. So, the hematological count indicates the features of seasons (Bett & Vinatea, 2009) [6]. The appropriate indications of these values interpret the species variations. Specifically, blood parameters provide physiological conditions and give details of prognosis. It's an aid for economic losses in the aquatic field (Francesco, 2018) [14]. Four teleost fishes such as *G.niger*, *M.cephalus*, *S.aurata*, and *D.labrax* were compared to bring out the similarities in hematological parameters, which suggested a clear knowledge of species concern (Fazio *et al*., 2013b) [12]. The research suggests the attribution of fish life, behavior, and environmental crisis.

The fundamental aspect, and for most immunity, is an innate immune system that acts as a defensive aspect. The lysozyme activates and destroys the invading pathogens and eliminates them from the body of fish. The NBT (Nitroblue tetrazolium) assay exhibits the production of oxidative radicals from monocytes and neutrophils in the defense mechanism (Balamurugan *et al*., 2012) [4]. The present study aims to present 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 this elaborate view, hematology, biochemical, and immunity play a vital role in indicating species physiology.

Material and Methods

Blood collection and preservation

Blood samples were collected from several species (20 species each) in the Kovalam, East Coast Road, Southeast coast of Tamil Nadu, India, during the summer and pre-monsoon seasons, each weighing between 120 to 175 g and measuring 18 to 25 cm in length. 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 hematological parameters

To determine the blood cells, first, the caudal fin was sterilized using a 70% alcohol swab to avoid contamination. Then the caudal vein was pricked with a needle. The blood was gently collected from the caudal vein using a disposable heparinized syringe. Eventually, the blood was stored in Eppendorf vials coated with EDTA anticoagulant. The improved Neubauer's hemocytometer 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) [10]. Anticoagulated blood was kept in the test tubes for one hour without being disturbed. The erythrocytes settled in the bottom and it was measured in mm/h. Hemoglobin (Hb) is estimated by a Sahli's haemoglobinometer. MCV, MCH, and MCHC were calculated by average values of Hb%, and Hematocrit (HCT) for the micro-hematocrit method was calculated using Dacie and Lewis (1977) [9]. For leukocyte count, a blood smear was prepared using Wright-Giemsa stain (Ghaffar *et al.*, 2017) [18] and the smear was examined by a light microscope under 100X magnification.

Determination of serum and immunological constituents

The Phosphomolybdate method and Lowry method were used to estimate the biochemical parameters glucose and protein respectively (Nimmy and Pawlin, 2018) [26]. The cholesterol and urea were determined using the standard method suggested by Francesco *et al*, (2012) [15]. The determination of the immunoglobulin IgM and IgG ELISA kit (EMP, M201, Microplate Reader) assay procedure was followed (Nimmy and Pawlin 2018) [26].

Lysozyme assay

The lysozyme assay method was followed by previous methods (Sakthivel *et al.*, 2012) [33]. It was a turbidometry assay for substrate 0.03% lyophilized Micococcus lysodeikticus was added into 0.05 mm sodium phosphate buffer pH value 5.9. Then 10µl of fish serum was mixed with 250µl of bacterial suspension in a U bottom duplicate well plate in microtiter (EMP, M201, Microplate Reader, USA), and the reduction in absorbance was determined at 520nm in 5min o incubation at 24^{0}C (LABINDIA analytical, UV 3200, UV-Vis Spectrophotometer). 0.001 per min of absorbance was measured in 1U for lysozyme.

Respiratory burst activity

For respiratory burst activity, the assay procedure was followed by (Balamurugan *et al*., 2012) [4] the reduction of NBT (nitro blue tetrazolium) was measured in formazan reactive oxygen radical. 40µl of blood was taken in a microtiter (EMP, M201, Microplate Reader, USA) incubated in a 1 hr water bath, and washed with 90µl PBS to remove non-adherence cells. Add 0.2% of NBT (100µl), and incubate the solution at room temperature. Then, 100% methanol ((100µl) was used as a fixative solution. After that 70% methanol was used to wash. 2N KOH with 140µl DMSO was used. 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) following Turkey's test was used for analysis. Statistically significant P values (<0.05) are considered.

Results

Effect of seasons on hematological parameters

In this report, hematology was investigated through White Blood Corpuscles (WBC), Red Blood Corpuscles (RBC), Hemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), and differential counts of thrombocytes, neutrophils, lymphocytes, eosinophils, and monocytes were examined and detailed results are interpreted in tables 1, 2 & 3. The values of RBC, WBC, Hb, and HCT are maximum in the summer, and MCV, MCH, and MCHC are also 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 & 3) in all three *M.cephalus*, *C.chanos*, and *A.arius* species. The highest leukocytes such as thrombocytes, neutrophils, lymphocytes, and eosinophils, and the lowest in the pre-monsoon (Tables 1, 2 & 3). While in between these three fishes, M.cephalus values are more than the other *C.chanos* and *A.arius* species in both seasons.

Table 1. Seasonal variations in hematological parameters of *M.cephalus*, in summer and pre-monsoon

Hematology	***M.cephalus***	
	Summer	**Pre-monsoon**
T.length (cm)	23.86±1.03*	22.13±1.04
T.weight (g)	171.22±35.42*	169.19±32.15
WBC (×10^6 µl)	28.09±1.04*	26.62±1.08

RBC (×10^3 µl)	2.56±0.25*	2.18±0.05
Hb (%)	39.04±1.39*	38.48±1.49
HCT (%)	28.17±0.13*	27.43±0.25
MCV (fL)	17.05±1.04	17.97±1.09*
MCH (pg)	149.08±3.81	150.17±3.22*
MCHC (%)	130.62±6.63	131.21±5.41*
ESR (mm/h)	0.48±0.25	1.02±0.43*
Thrombocytes (%)	30.08±4.62*	29.94±4.03
Monocytes (%)	10.68±1.06*	10.02±1.06
Lymphocytes (%)	81.01±2.15*	79.57±1.05
Neutrophils (%)	12.22±1.60*	11.87±1.04
Eosinophils (%)	1.08±0.33*	0.95±0.09

Note: Asterisk (*) indicates significant variation (P<0.05)

Table 2. Seasonal variations in hematological parameters of *C.chanos*, in summer and pre-monsoon

Hematology	***C.chanos***	
	Summer	**Pre-monsoon**
T.length (cm)	22.36±1.17*	21.61±1.27
T.weight (g)	163.03±33.32*	162.15±32.13
WBC (×10^6 µl)	26.43±1.48*	25.32±1.09
RBC (×10^3 µl)	2.62±0.53*	2.54±0.29
Hb (%)	36.82±1.68*	35.62±1.38
HCT (%)	27.08±0.11*	26.03±0.07
MCV (fL)	16.75±1.51	17.05±1.03*
MCH (pg)	182.36±10.06	183.13±9.43*
MCHC (%)	129.51±13.01	130.04±12.37*
ESR (mm/h)	0.52±0.18	1.16±0.28*
Thrombocytes (%)	29.13±5.01*	28.60±4.43
Monocytes (%)	10.24±1.17*	9.12±1.03
Lymphocytes (%)	80.76±2.07*	78.41±1.33
Neutrophils (%)	12.08±1.19*	11.69±1.08
Eosinophils (%)	1.09±0.18*	1.01±0.03

Note: Asterisk (*) indicates significant variation (P<0.05)

Table 3. Seasonal variations in hematological parameters of ***A.arius***, in summer and pre-monsoon

Hematology	*A.arius*	
	Summer	Pre-monsoon
T.length (cm)	22.62±1.08*	20.76±1.38
T.weight (g)	168.72±35.04*	166.42±31.02
WBC (×10^6 µl)	28.30±1.57*	27.12±1.25
RBC (×10^3 µl)	2.01±0.08*	1.87±0.02
Hb (%)	36.05±1.43*	35.05±1.03
HCT (%)	26.72±0.16*	25.62±0.53
MCV (fL)	16.49±1.17	17.42±1.54*
MCH (pg)	173.14±3.22	174.68±3.21*
MCHC (%)	126.09±12.27	127.76±13.66*
ESR (mm/h)	0.45±0.27	1.05±0.08*
Thrombocytes (%)	30.45±5.38*	29.45±4.67
Monocytes (%)	9.92±1.35*	9.18±1.02
Lymphocytes (%)	80.52±2.19*	79.84±2.03
Neutrophils (%)	12.03±1.63*	11.92±1.16
Eosinophils (%)	1.08±0.41*	0.89±0.18

Note: Asterisk (*) indicates significant variation (P<0.05)

Effect of seasons on biochemical and immunological parameters

In biochemical parameters, the serum glucose, protein, cholesterol, and urea content are estimated seasonally. Glucose and protein were recorded as an increase in summer in all three species. By contrast, 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 (Tables 4, 5 & 6).

Table 4. Seasonal variations in serum biochemical & immunological parameters of *M.cephalus* in summer and pre-monsoon

Biochemical	*M.cephalus*	
	Summer	Pre-monsoon
Glucose (mg/dl)	88.04±1.68*	85.73±1.15
Protein (mg/dl)	5.82±0.47*	4.08±0.33
Cholesterol (mg/dl)	189.04±2.42	191.01±2.04*
Urea (mg/dl)	6.62±0.16	6.85±0.16*
Immunological		
IgM	8.53±1.08*	7.84±1.02

IgG	7.77±1.23*	6.53±1.21

Note: Asterisk (*) indicates significant variation (P<0.05)

Table 5. Seasonal variations in serum biochemical & immunological parameters of *C.chanos* in summer and pre-monsoon

Biochemical	***C.chanos***	
	Summer	**Pre-monsoon**
Glucose (mg/dl)	85.18±1.53*	82.49±1.02
Protein (mg/dl)	5.29±0.45*	4.16±0.42
Cholesterol (mg/dl)	182.17±0.08	188.08±0.17*
Urea (mg/dl)	6.31±0.08	7.08±0.05*
Immunological		
IgM	7.92±1.32*	7.01±1.05
IgG	7.82±1.03*	6.82±0.83

Note: Asterisk (*) indicates significant variation (P<0.05)

Table 6. Seasonal variations in serum biochemical & immunological parameters of *A.arius* in summer and pre-monsoon

Biochemical	***A.arius***	
	Summer	**Pre-monsoon**
Glucose (mg/dl)	85.13±1.72*	82.63±1.38
Protein (mg/dl)	5.36±0.38*	4.29±0.26
Cholesterol (mg/dl)	185.05±2.32	189.43±2.02*
Urea (mg/dl)	5.83±0.12	6.12±0.09*
Immunological		
IgM	7.50±1.62*	6.95±1.06
IgG	7.49±1.14*	6.24±1.02

Note: Asterisk (*) indicates significant variation (P<0.05)

Furthermore, in all three selected fishes, IgM and IgG were significantly higher in summer and lower in pre-monsoon (Tables 4, 5 & 6) 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 the data is illustrated in tables (Tables 7, 8 & 9).

Table 7. The level of WBCs & Lysozyme activity & NBT activity of *M.cephalus* in summer and pre-monsoon

Activities	M.cephalus	
	Summer	**Pre-monsoon**
T.leukocytes	28.09±1.04*	26.62±1.08
Lysozyme activity	1218±89	1253±84*
NBT activity	0.25±0.01	0.76±0.04*

Note: Asterisk (*) indicates significant variation (P<0.05)

Table 8. The level of WBCs & Lysozyme activity & NBT activity of *C.chanos* in summer and pre-monsoon

Activities	C.chanos	
	Summer	**Pre-monsoon**
T.leukocytes	26.43±1.48*	25.32±1.09
Lysozyme activity	1213±91	1249±94*
NBT activity	0.26±0.07	0.68±0.07*

Note: Asterisk (*) indicates significant variation (P<0.05)

Table 9. The level of WBCs & Lysozyme activity & NBT activity of *A.arius* in summer and pre-monsoon

Activities	A.arius	
	Summer	**Pre-monsoon**
T.leukocytes	28.30±1.57*	27.12±1.25
Lysozyme activity	1208±77	1262±80*
NBT activity	0.24±0.21	0.72±0.18*

Note: Asterisk (*) indicates significant variation (P<0.05)

Discussion

All over the world, seasonal changes cause a significant impact on most species, and the effect of seasonal fluctuation is well defined in hematological variation in fishes. The present observation reveals the seasonal changes and effects on hematological parameters of *M.cephales*, *C.chanos*, and *A.arius*. The study of hematology has sensitive and effective parameters in fish and is more important in evaluating pathological, and physiological changes caused by pollution in an aquatic ecosystem. Therefore, hematology is a huge 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) [36]. As a result, hematological studies are effective for determining reaction and health status in aquatic contamination exhibits (Pimpao *et al*., 2007) [29]. The seasons, gender, mass, propagation, health, water temperature, environment, nutrition, and stress may be affected by hematological indices (Hrubec *et al*., 2001) [21]. The hematological 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 increased hematology was observed by Faheem *et al*, (2021) [10] in *L.rohita*. The concentration of Hb, HCT, ESR, thrombocytes, and neutrophils were significantly higher in the summer while they declined in the pre-monsoon. Similar findings were noted in

M.armatus (Narejo *et al*., 2002) [25]. In pre-monsoon, non-significant parameters are seen in hematology. More deviations were observed in selected species in summer as well as pre-monsoon (Gupta and Mishra, 2012) [19]. A review of blood counts and biochemical parameters in three species of the Cauvery River was incorporated into our report (Mohan and Dhanapalan, 2015) [24]. 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, which the fish health affected subsequently (Faheem *et al*., 2021) [10].

Serum biochemical parameters mainly reflect fish maturity, and they monitor significant changes in fish health and water quality related to the ecosystem. Serum biochemical differences vary significantly in each species and may be influenced by seasons, temperature, food habits, sex, and age of the species (Jawad et al., 2004) [22]. The serum glucose and protein were in an increased state in the 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) [34]. Moreover, cholesterol and urea values were increased during the pre-monsoon while the protein and glucose were decreased. These levels of cholesterol indications due to lipid metabolism and liver dysfunction were closely related to previous reports (Kavadias *et al*., 2004) [23]. Hematological and biochemical parameters are tools for monitoring fish health in all seasons. The main aim of studying the seasonal variations in fish is to determine the seasonal fluctuation of the hematological parameters. Different aspects of fish depended on the temperature in various seasons (Shahjahan et al., 2018) [35]. The main factor in immunity is water temperature. Water temperature controls the immunity of the fish. The immune patterns of *M.cephalus*, *C.chanos*, and *A.arius* the IgM and IgG levels higher in the summer which was lower in the pre-monsoon. During summer, the immunoglobulins were higher due to the spawning season for selected fish. Meanwhile, pre-monsoon immunity factors do not need to be linked to water temperature because they were found to be associated with seasonal changes in immune activities in *C.carpio* (Saha *et al*., 2003) [32]. The IgM and IgG imply a greater significance in the immune system of teleost fish.

The bactericidal enzyme of innate immunity is lysozyme. It overreacted in the acute phase of protein during stress conditions and infections, acting as a functional aspect against contagious diseases. Here, the lysozyme was moderately highest in the summer and declined in the pre-monsoon. So, according to our findings, the level of lysozyme in fish is unaffected by temperature or 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 a 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 maximum during pre-monsoon.

Conclusion

This study was undertaken and 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 fish. Likewise, fish blood parameters are also considered to interpret fish health. For further studies, our report suggested that the seasons are taken into consideration to monitor water changes.

Acknowledgments

The authors thank CAS Marine Biology, Faculty of Marine Sciences, Dean and Director authorities of Marine Sciences, Annamalai University. We thank the financial support of AU/DRD/RUSA-2.0/R&I/Project Proposal/Field-5/6/2021.

References

1. Ali HA, Rani VJ. Effect of phosalone on haematological indices in the tilapia, *Oreochromis mossambicus*. *Turk. J. Vet. Anim. Sci*,2009:33:407-411.
2. Ambreen F, Javed M. Pesticide mixture induced DNA damage in peripheral blood erythrocytes of freshwater fish, *Oreochromis niloticus. Pak. J. Zool*,2018:50:339-346. https://doi. org/10.17582/journal.pjz/2018.50.1.339.346.
3. Ahmed, Imtiaz, Reshi, Quseen Mushtaq, Fazio, Francesco. *The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquaculture International,*2020. doi:10.1007/s10499-019-00501-3.
4. Balamurugan S, Deivasigamani B, Kumaran S, Sakthivel M. Seasonal changes effect on Innate immunity and histology of wild catfish *Arius arius* (Hamilton, 1822) in Parangipettai, south East coast of Tamil Nadu, India. *Theriogenology Insight*,2012:2(3):197-207.
5. Begum A, Minar MH, Sarower-E-Mahfuj M, Begum M. Monthly variation of biochemical composition of gonia (*Labeogonius*) collected from Bangladeshi water. *International Journal of Life Sciences Biotechnology and Pharma Research*,2013:2(2):227-232.
6. Bett C, Vinatea L. Combined effect of body weight, temperature and salinity on shrimp *Litopenaeus vannamei* oxygen consumption rate. *Brazilian Journal of Oceanography*,2009:57(4):305-314.
7. Çelik ES. Çanakkale Bo˘gazı'ndan Avlanan ˙Iskorpit Balı ˘ gı (*Scorpaena porcus* Linnaeus, 1758)'nın Kan Glukoz Düzeyindeki Aylık De˘ gi¸smeler. Su. Ürünleri. Dergisi,2005:22:115–118.
8. Çelik ES. Çanakkale Bo˘gazı'ndan Avlanan ˙Iskorpit Balı ˘ gı (*Scorpaena porcus* Linnaeus, 1758)'nın Kan Glukoz Düzeyindeki Aylık De˘ gi¸smeler. Su. Ürünleri. Dergisi,2004:22:115–118.
9. Dacie JV, Lewis SM. '*Practical hematology*' 8th ed. Longman group Ltd. London,1977:22-68.
10. Faheem S, Kalhoro H, Narejo NT, Chandio MH, Samina M, Rashid S, Abbas G. Fish health assessment in relation to sex and season: haematological variations and serum biochemical constituents of *Labeo rohita* (Hamilton, 1822) from Kotri Barrage near Jamshoro, Sindh, Pakistan. *Sarhad Journal of Agriculture*,2021:37(3):1043-1049.
11. Fazio F, Marafioti S, Sanfilippo M, Casella S, Piccione G. Assessment of immune blood cells and serum protein levels in *Mugil cephalus* (Linnaeus, 1758), *Sparus aurata* (Linnaeus, 1758) and *Dicentrarchus labrax* (Linnaeus, 1758) collected from the Thyrrenian sea coast, Italy. *Cahiers De Biologie Marine*,2016:57:235-240.
12. Fazio F, Marafioti S, Arfuso F, Piccione G, Faggio C. Comparative study of the biochemical and haematological parameters of four wild Tyrrhenian fish species. *Vet. Med*,2013b:58:576–581.

13. Ferri J, Mati´c-Skoko S, Cˇ ož-Rakovac R, Strunjak-Perovic´I. Beer Ljubi´c B, Topi´c Popovi´c N. Assessment of Fish Health: Seasonal Variations in Blood Parameters of the Widely Spread Mediterranean Scorpaenid Species, *Scorpaena porcus*. *Appl. Sci*,2022:12:4106. https://doi.org/10.3390/app12094106.
14. Francesco Fazio, Fish hematology analysis as an important tool of aquaculture: A review. Aqua,2018:doi:10.1016/j.aquaculture.2018.10.030.
15. Francesco F, Satheeshkumar P, Senthil Kumar D, Caterina F, Giuseppe P. A Comparative study of hematological and blood chemistry of Indian and Italian Grey Mullet (*Mugil cephalus Linneaus* 1758). *HOAJ Biology*,2012:1:5. http://dx.doi.org/10.7243/2050-0874-1-5.
16. Ghaffar A, Hussain R, Abbas G, Akram K, Latif H, Khan A. Assessment of genotoxic and pathologic potentials of fipronil insecticide in *Labeo rohita* (Hamilton, 1822). *Toxin Reviews*,2019:1-12. https://doi.org/10.1080/15569543.2019.1684321.
17. Ghaffar A, Hussain R, Abbas G, Kalim M, Khan A, Ferrando S, Gallus L, Ahmed Z. Fipronil (Phenylpyrazole) induces hemato-biochemical, histological and genetic damage at low doses in common carp, *Cyprinus carpio* (Linnaeus, 1758). *Ecotoxicology*,2018:27:1261-1271. https://doi.org/10.1007/s10646- 018-1979-4.
18. Ghaffar A, Hussain R, Abbas G, Ahmad MN, Abbas A, Rahim Y *et al*. Sodium arsenate and/ or urea differently affect clinical attributes. *hematobiochemistry and DNA damage in intoxicated commercial layer birds. Toxin Rev*,2017:37:206-215.
19. Gupta S, Mishra P. Haematological variation in cat fish. *Asian J. Anim. Sci*.2012:7(1):19-22.
20. Habib SS, Fazio F, Naz S, Piccione FAG, Rehman HU, Achakzai MNU, Rind KH, Rind AR. Seasonal variations in haematological parameters and body composition of *Labeo rohita* (Rohu) and *Cirrhinus mrigala* (Mrigal carp) in River Indus, District Dera Ismail Khan, Pakistan. *Turkish Journal of Fisheries and Aquatic Sciences*,2021:21:435-441. http://doi.org/10.4194/1303-2712-v21_7_01.
21. Hrubec T, Smith S, Robertson J. Age-related changes in hematology and plasma chemistry values of bird striped bass (Morone chrysops, Morone saxatilis). *Vet.Clin.Pathol*,2001:30:8-15.
22. Jawad LA, Al-Mukhtar MA, Ahmed HK. The relationship between haematocrit and some biological parameters of the Indian shad, *Tenualosa ilisha* (Family Clupidae). *Anim Biodivers Conserv*,2004:27:478–483.
23. Kavadias S, Castritsi-Catharios J, Dessypris A. Annual cycles of growth rate, feeding rate, food conversion, plasma glucose and plasma lipidsin the population of European sea bass (*Dicentrarchus labrax*) farmed in floating marine cages. *J Appl Ichthyol*,2004:19:29–34.
24. Mohan A, Dhanapalan S. Assessment of the haematological and serum biochemical parameters of three commercially important freshwater fishes in river Cauvery Velur, Namakkal district, Tamil Nadu, India. *Int. J. Fish. Aquac. Stud*,2015:4(1):155-159.

25. Narejo NT, Rashid MM and Rahmatullah SM. Seasonal and sex related haematological variations of the freshwater spiny eel, *Mastacembelus armatus* (Lacepede) reared in the cemented cistern, Bangladesh. *J. Fish. Res*,2002:6(2):141-147.
26. Nimmy MV, Pawlin Vasanthi Joseph. Impact of Lead nitrate on the Haematological, Biochemical and immunological response of the Freshwater Fish Cirrhinus mrigala. *International Journal of Science and Research Methodology*,2018:8(3):380-400.
27. Norouzi M, Bagheri M. The chemical composition of golden grey mullet *Liza aurata* in southern Caspian Sea during sexual rest and sexual ripeness. *Aquaculture, Aquarium, Conservation & Legislation*,2015:8(4):517-525.
28. Panigrahi AK, Choudhury N, Tarafdar J. Pollutional impact of some selective agricultural pesticides on fish *Cyprinus carpio*. IMPACT: *Int. J. Res. Appl. Nat. Soc. Sci*,2014:2:71-76.
29. Pimpao CT, Zampronio AR, De Assis HS. Effects of deltamethrin on hematological parameters and enzymatic activity in *Ancistrus multispinis* (Pisces, Teleostei). *Pest. Biochem. Phys*,2007:88:122–127. https://doi.org/10.1016/j. pestbp.2006.10.002.
30. Rudneva I, Skuratovskaya E, Kuzminov N, Kovyrshina T. Age composition and antioxidant enzyme activities in blood of Black Sea teleosts. *Comp. Biochem. Physiol. C Toxicol. Pharmacol*,2010:151:229–239.
31. Rudneva I, Skuratovskaya E. Gender peculiarities of blood antioxidant enzyme activity of some Black Sea coastal fish species. *J. Ichthyol*,2009:49:119–122.
32. Saha NR, Usami T, Suzuki Y. Seasonal changes in the immune activities of common carp (*Cyprinus carpio*). *Fish Physiology and Biochemistry*,2003:26:379–387.
33. Sakthivel M, Deivasigamani B, Kumaran S, Balamurugan S, Rajasekar T. Seasonal variation in immune organs and immune response of catfish *Arius maculatus* in Parangipettai coastal area. *Journal of Chemical and Pharmaceutical Research*,2012:4(7):3342-3348.
34. Satheeshkumar P, Ananthan G, Senthil Kumar D, Jagadeesan L. Hematology and biochemical parameters of different feeding behaviour of teleost fishes from Vellar estuary, India. *Comparative Clinical Pathology*,2011. DOI: 10.1007/s00580-011-1259-7.
35. Shahjahan MD, Helaluddin MD, Bain V, Haque MM. Increased water temperature altered hematobiochemical parameters and structure of peripheral erythrocytes in striped catfish, Pangasianodon hypophthalamus. *Fish Physiol Biochem*,2018:44:1309-1318.
36. Tahir R, Ghaffar A, Abbas G, Turabi TH, Kausar S, Du X, Naz S, Jamil H, Samra S. Riaz and S.S. Abdelgayed. Pesticide induced hematological, biochemical and genotoxic changes in fish: *A Review. Agrobiol. Rec*, 2021:3:41-57.
37. Yadav KK, Gupta N, Kumar V, Choudhary P, Khan SA. GIS-based evaluation of groundwater geochemistry and statistical determination of the fate of contaminants in shallow aquifers from different functional areas of Agra city, India: *levels and spatial distributions. RSC Adv*, 2018a:8:15876-15889. https://doi.org/10.1039/C8RA00577J.