



A Comparative study of hematological and blood chemistry of Indian and Italian Grey Mullet (*Mugil cephalus* Linnaeus 1758)

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Abstract

Background: Blood hematological and serum biochemistry parameters are often used to assess the health status and as stress indicators in fishes. In the present study was undertaken to comparative study of hematological and blood chemistry of Indian and Italian Grey Mullet (*Mugil cephalus*) to establish baseline values. Fifteen sexually immature and disease-free Indian wild fish (26.25 ± 0.23 cm total length, 347.55 ± 14.27 g weight) and 15 Italian fish (31.53 ± 1.08 cm total length, 416.5 ± 14.56 g weight) were examined.

Results: Statistical analysis revealed that differences in hematological and biochemical parameters between two region mullet fish were significant ($P < 0.01$). The RBC/WBC level increased due to the decrease in WBC during the comparative study.

Conclusions: The study showed that the environmental conditions significantly impacted the status of the fish. It is suggested that these physiological parameters can be conveniently employed as health monitoring tools in fish culture practices. Biochemical parameters also are indicative of the habits of fishes, and can be used for confirming the maturity and monitoring any changes in the quality of waters.

Background

Mullets have worldwide distribution and inhabit tropical and temperate waters and few spend their lives in freshwater [1]. They are grouped taxonomically in the family Mugilidae includes 17 genera and 72 species in the world [2]. Mullet (*Mugil cephalus*) are Perciform species, which feeds mainly on zooplankton, benthic organisms and detritus, and was chosen because it possesses several characteristics required in an estuarine sentinel species, such as the extreme salinity tolerance [3]. Understanding the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology [4,5]. Hematological and biochemical parameters are being used as indicators in the measurement of health conditions and toxicological symptoms of organisms [6]. While providing information about the health status of organisms, these parameters may also indicate abnormal environmental conditions [7]. Information about the existence, status and degree of possible sickness in organisms can be rapidly obtained by with use of hematological and biochemical parameters. One of the difficulties in assessing the state of health of natural fish population has been the paucity of reliable references of the normal condition [5,8]. Although fish haematology continues to offer the potential of a valuable tool, progress in establishing normal range values for blood parameters has been sparse and literature in this area is isolated and often incomplete [5]. Despite advances in fish medicine in recent years, interpretation of fish haematology is often troubled by a lack of meaningful reference values and the bewildering diversity of fish species [9]. Only a few normal values for a small

number of haematological parameters have been established for some teleosts, but these values range widely due to the lack of standardized collecting and measuring techniques. Haematological studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. A multitude of intrinsic and extrinsic factors cause normal and abnormal variations in haematologic data [9] such as species and strain [10], temperature [10,11], age [12], stress [14]; photoperiod [15], nutritional state [12, 16], the cycle of sexual maturity, health condition [17], and water quality. The main objective of this present study was to comparative investigation on haematological and biochemical parameters on *M. cephalus*, captured in two different habitat Faro lake (Italy) and Vellar estuary (India). These data can be used to monitor the physiological status of individual species to assess in wild and in aquaculture.

Material and methods

Study area

Faro Lake

Cape Peloro is a brackish system located in the north-eastern corner of Sicily (Lat $38^{\circ}15'57''$ N; Long $15^{\circ}37'50''$ E). It consists of two basins, Ganzirri and Faro, communicating with the Tyrrhenian Sea by English canal and connected to each other by Margi canal [18].

Vellar Estuary

Healthy marine teleost fishes were collected from Vellar estuary (Lat. $11^{\circ}29'$ N and Long $79^{\circ}46'$ E), Parangipettai of southeast coast of Tamil Nadu, India. The estuary is seasonally bar-built and semi-diurnal type flows eastwards and empties into the Bay of Bengal at Parangipettai on southeast coast of India, carrying the wastes from the adjacent agriculture lands and industries in addition to domestic municipal and distillery effluents.

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Sampling and analytical methods

In this present study 30 *M. cephalus* were investigated in two different habitat Faro Lake (Italy) and Vellar estuary (India). They were divided into two equal groups on the basis of the site of collection. 15 fish were caught in Faro Lake – Sicily (group A) and 15 were caught in Vellar estuary (India) (group B). All fish were caught with bottom-set nets and blood samples were immediately collected. All fish samples were collected from 2007 to 2008. At the end of blood sampling on all subject weight and length were recorded (Table 1). On the basis of their weight and length all fish were considered sexually mature and with age between two and four years [19]. Water sampling was carried out in the same date of fish sampling, in three stations points of Faro Lake and Vellar Estuary. The three stations on the each location were selected randomly and the distances among them were about 3 meter to 1.5 Km. In Faro lake, water samples collected by Niskin bottle (General Oceanics, Inc.-Miami, Florida) for sampling and a multiparametric probe YSI 85 System for temperature, salinity, dissolved oxygen (DO) and pH. In Vellar estuary from the collection site, the water quality dissolved oxygen was estimated by Winkler's methods [20], salinity by an Erma hand refractometer (Tokyo). The pH and water temperature were measured by using pH tester pen (Japan) and thermometer, respectively.

Blood samples were collected by caudal vein/ direct heart puncture by using a sterile plastic syringe (2.5 mL) and transferred into 2 different tubes, one (Miniplast 0.5 ml, LP Italiana Spa, Milano) containing EDTA (1.26 mg/ 0.6 mL) as an anticoagulant agent and the other without EDTA. The blood samples were collected in EDTA tubes were used for the determination of haematological profile. Heparin sodium (1%) was used as an anticoagulant [21]. The collected blood samples were immediately subjected to hematological analysis. Evaluation of the haemogram involves the determination of the Red Blood Count (RBC), Haematocrit (Hct), Hemoglobin concentration (Hgb), White Blood Cell Count (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC).

The bloods were diluted with appropriate diluting fluids for RBC and WBC counts and were determined using improved Neubauer haemocytometer and calculated [22,23]. Replicated counts were made for each blood samples to minimise the error. Hematocrit was determined by microhematocrit centrifugation. Microcapillary

tubes were filled, plugged with clay, and centrifuged at 19,000g for 5 minutes. Measure the length of the columns containing packed red cells, and packed red cells plus supernatant. The calculation of hematocrit is as follows: (packed red cells/packed red cells plus supernatant)/100%. Haemoglobin concentration was measured with Hb test kit (Roach GmbH Mannheim, Germany) using the cyanmethemoglobin method [24] and Sahili Haemoglobinometer. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were calculated indirectly by the above direct parameters values using standard formulas.

For the assessment of glucose on whole blood, a portable blood glucose analyzer (ACCU-Chek Active, Roche Diagnostics GmbH, Mannheim, Germany). On serum samples obtained from blood samples without EDTA by centrifugation for 10 min at 3000 rpm, total protein, cholesterol and urea were determined by means of commercial kits (BioSystem, Barcelona, Spain) using on UV spectrophotometer (Slim, SEAC, Florence, Italy). In Indian mullet, Biochemical estimation of blood glucose, protein, cholesterol, and urea were determined by following standard methods [25,26,27,28].

Statistical Analysis

Differences in haematological parameters between the two study stations marine teleost fish were statistically analyzed by Unpaired T-test. Mean and standard error (SEM) were calculated for each parameter. All these statistical analyses were performed using the statistical software Prism v. 4.00 (Graphpad Software Ltd., USA, 2003).

Results

Table 1 shows the physico chemical parameters of both sampling stations Faro Lake (Italy) and Vellar Estuary (India). The temperature of Indian station was higher than Italian with a statistical *p* value of (*p*<0.05). The other parameters such as pH, salinity and DO were high in Italian station than India and with *p* value (*p*< 0.1, *p*< 0.001 and *p*< 0.01) respectively. Table 2 indicates the biometric parameters of Grey Mullet (*M. cephalus*) collected in both stations. 15 sexually immature and disease-free Indian wild fish (26.25 ± 0.23 cm total length, 347.55±14.27 g weight) and 15 Italian fish (31.53 ± 1.08 cm total length, 416.5 ± 14.56 g weight) were examined. Among these Faro lake fishes were with high mean length (31.53±1.08cms) and weight

Table 1. Descriptive statistics of biometric data, together the statistical significant, in 30 *Mugil cephalus* from Faro Lake (Italy) and Vellar estuary (India).

Site of collection	Biometric parameters							
	Length (cm)				Weight (g)			
	Mean±SEM	Min	Max	CV (%)	Mean±SEM	Min	Max	CV (%)
Faro Lake (Italy) (n=15)	31.53±1.08	19.50	36	13.28	416.50±14.56	340	510	13.54
Vellar estuary (India) (n=15)	26.25±0.23*	25.00	28.1	3.40	347.55±14.27*	235	410	15.90

Significance: *Vs Faro Lake (Italy)

Table 2. Mean values ± SEM of physico-chemical factors of water investigated in the two sampling sites.

Parameters	Faro Lake (Italy)	Vellar estuary (India)	P value
T (°C)	25.30±0.29	31.83±2	<i>P</i> <0.05
pH	8.11±0.013	7.8±0.24	<i>P</i> <0.1
Salinity (ppt)	33.43±0.48	26.16±1.43	<i>P</i> <0.001
DO ml l ⁻¹	5.89±0.033	4.48±0.30	<i>P</i> <0.01

T=Temperature; DO=Dissolved Oxygen

(416.50±14.56 gms). Both the biometric parameters were significant with Vellar estuary samples. The haematological parameters of *M. cephalus* of Faro lake (Group A) and Vellar estuary (Group B) were expressed in Table 3. RBC, RBC/WBC ratio and Hct % were all higher in Group A than B with a P value of 0.0001. Remaining parameters such as WBC, MCH, MCHC and MCV were elevated in Group B than A with p value of 0.0001 and 0.1 respectively. The biochemical parameters detected in the serum of both group fishes were shown in Table 4. Among the organic constituents proteins, glucose, urea and cholesterol were found higher in Group A and are statistically significant ($p < 0.0001$ and $p < 0.2$ in urea).

Discussion

The physiological condition in fishes required for their selection as broodfish can be determined by hematological studies. The blood constituents in teleost fishes are influenced by factors like temperature ecological habitat, food selection and mode of life. Therefore, it is difficult to establish any normal values for the class as a whole. But, if data are collected for different species as well as within species under different conditions some normal ranges of values can be arrived at, which can form a valuable diagnostic aid in fisheries [29]. Studies of blood parameters had proven to be a valuable approach for analysing the health status of fish and help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment [30,31]. The ranges of normal values of the key biochemical parameters are still undefined for same species living in different habitat. In the aquatic habitat, the fish homeostatic system is continuously affected by the changes of the level of salinity, temperature, pH, oxygen concentration [32]. The physiological response to environmental variations such as salinity, temperature and DO fluctuations in aquatic system has been investigated in fresh and marine water species [33,34]. In our study significant differences were observed in some haematological and biochemical parameters between groups A and B from Italian and Indian sampling sites respectively (Table 3 and 4). In particular our results showed that RBC values,

Hct and RBC/WBC ratio were higher in group A compared to group B while Hgb, WBC, MCH and MCHC values were lower in group A compared to group B. As fish are very susceptible to environmental physical and chemical changes which may be reflected in their blood components [34], the significant differences in some haematological and biochemical parameters in the two groups of *M. cephalus* could be attributed to different habitat conditions. In fact, as shown in Table 2 significant differences in physico-chemical factors of water, investigated in the two sampling sites were observed. In particular salinity and DO values of Faro Lake were higher than those of Vellar estuary, while temperature was lower in the Italian site compared to Indian site. Salinity variation between the two sampling sites could justify the higher Hct values in group A compared to group B in according with a study on rainbow trout [35].

Our results showed that when water temperature increased and salinity and DO decrease RBC count decreased and Hb increased. It has been observed that blood parameters such as Hct, Hb and RBC count are related to environmental factors such as water temperature and salinity. Moreover, the relationship between haemoglobin and oxygen shows adaptations not only to environmental conditions but also to metabolic requirements, both of which govern oxygen availability and transport to tissue [36]. These adaptations may involve quantitative changes in total Hb content, or qualitative changes in Hb-oxygen-binding properties, and may appear both at the inter- and intra- specific level [37].

The differences in WBC count in the two groups of *M. cephalus* studied here could be the result of different body size of fish as there was statistical difference in biometric data of the two fish groups as it is known that differences in WBC may be attributed to many factors, both biotic (such as age, season, maturity, pathogens) and abiotic (including water temperature, pH, dissolved oxygen content) and in particular to stress [38]. There is an inverse relationship between WBC and RBC counts observed in the two groups. High RBC count perhaps lessens the requirement for large number of WBC [5,38]. Moreover, the lower WBC count consequently leads to higher RBC/WBC ratio in group A compared to group B. The variations

Table 3. Mean values ± SEM of haematological parameters obtained into two experimental groups (abbreviations are explained in the text).

Haematological parameters	Faro Lake (Italy) (group A)			(India) (group B)		Vellar estuary	P value
	Min	Max	Mean ± SEM	Min	Max		
RBC (x106 µ/ L)	2.21	4.47	3.53±0.16	2.32	2.7	2.51±0.026	$P < 0.0001$
WBC (x103 µ /L)	16.30	21.00	18.30±0.40	27.56	29.21	28.14±0.13	$P < 0.0001$
Hgb (g/dL)	5.70	13.30	10.65±0.60	35.4	38.5	37.03±0.21	$P < 0.0001$
RBC/WBC (%)	1.07	2.39	1.94±0.08	0.076	0.088	0.82±0.04	$P < 0.0001$
Hct (%)	21.0	50.0	39.60±2.10	27.56	28.70	28.06±0.11	$P < 0.0001$
MCV (fl)	95.02	138.6	111.5± 2.45	10.71	12.3	115.6±1.48	$P < 0.1$
MCH (pg)	25.44	34.50	29.83±0.60	140	156	148.4±1.17	$P < 0.0001$
MCHC (g/ dL)	21.09	31.43	26.88±0.62	117	135	129.2±1.20	$P < 0.0001$

Table 4. Mean values ± SEM of biochemical parameters obtained into two experimental groups.

Parameters	Faro lake (group A) Mean ± SEM	Vellar estuary (group B) Mean ± SEM	P value
Total proteins (mg/dL)	2.05±0.16	3.58±0.041	$P < 0.0001$
Glucose (mg/dL)	70.80±1.51	84.05±1.06	$P < 0.0001$
Cholesterol (mg/dL)	175.7±2.79	195.4±2.88	$P < 0.0001$
Urea (mg/dL)	5.44±0.96	6.58±0.10	$P < 0.2$

of MCH and MCHC values as effect of different habitat conditions are controversial. In our study high salinity and temperature lead to a lower MCH and MCHC values while [39] in pike perch noted an increase of these parameters with an increased salinity level.

Significant variations in some blood biochemical parameters between group A and B were observed in this study. In particular, our results showed lower concentrations of total proteins, glucose and cholesterol in group A than group B. These parameters are considered to be major indices of the health status of teleosts. Increased concentrations of total proteins can be caused by structural liver alterations reducing aminotransferase activity, with concurrent reduced deamination capacity and impaired control of fluid balance [40]. Elevated levels of cholesterol indicate disorders of lipid and lipoprotein metabolism, especially liver disease [41]. However the differences in blood biochemical parameters found in the two experimental groups of this study were attributed to the different habitat, in fact it is known that the ranges of serum biochemistry can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age and sex of the fish [36].

Conclusions

The results of this study provide the knowledge of the characteristics of haematological and biochemical parameters of *M. cephalus* from two different habitats and show that many physico-chemical factors of water influence the ranges of haematology and of serum biochemistry of fish within same species suggesting that blood parameters may therefore be a value in monitoring the effects of habitat changes on fish biology and fish culture practices.

Competing interest

The Authors declare that they have no competing interest.

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