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A comparative evaluation of hematological and biochemical parameters between the Italian mullet *Mugil cephalus* (Linnaeus 1758) and the Turkish mullet *Chelon auratus* (Risso 1810)

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Abstract: This study aimed to carry out a comparative evaluation of the hematological profile (erythrocyte count, hemoglobin concentration, hematocrit, and Wintrobe index parameters such as mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration), in addition to certain biochemical parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, serum total protein, albumin, glucose, total cholesterol, and triglycerides), in the Italian mullet *Mugil cephalus* (Linnaeus 1758) and the Turkish mullet *Chelon auratus* (Risso 1810). Accordingly, two groups of fish were used in this study: 30 flathead grey mullets, *M. cephalus*, caught in Lake Faro, Italy, and 30 golden grey mullets, *C. auratus*, caught in the estuarine channel system of Köyceğiz–Dalyan, Turkey. Statistical evaluations (unpaired t-test) revealed that there were no significant differences in weight or total length values between the two mullet groups. However, the differences between all the evaluated blood parameters (except alanine aminotransferase) between the Italian and the Turkish mullet were significant ($P < 0.0001$). The present study demonstrated that there were significant hematological and biochemical differences between the Italian (*M. cephalus*) and the Turkish (*C. auratus*) mullet. Our findings also contribute to expanding the knowledge on the hematology and biochemistry of two different species of mullet originating from two different habitats. Since the evaluation of blood parameters represents an essential tool in examining the effects of environmental conditions on fish physiology and, consequently, on fish health status, further research in this field is encouraged and would be very useful.

Key words: Biochemical parameters, *Chelon auratus*, hematological profile, Italy, *Mugil cephalus*, Turkey

1. Introduction

The flathead grey mullet, *Mugil cephalus* (Linnaeus 1758), and the golden grey mullet, *Chelon auratus* (Risso 1810), are two species of fish belonging to the family Mugilidae with commercial importance in tropical and warm temperate estuaries (Blaber, 1997) and an important ecological function in food chains (Cardona, 2001). Unfortunately, nowadays, the status of mugils is very fragile due to continuous pollution from agricultural and industrial activities and also high fishing pressure. In fact, *M. cephalus* is a species that is becoming rare and endangered (Glamuzina and Bartulović, 2010). Several authors have previously published important studies on this fish (Cardona, 2006; Fazio et al., 2014, 2015, 2019; Gisbert et al., 2016; Parrino et al., 2018). It is a euryhaline and eurythermal species of considerable commercial importance that consistently contributes to fisheries of estuarine and coastal regions in numerous countries (Saleh, 2006; Whitfield et al., 2012). Moreover, in terms of aquaculture diversification, the grey mullet is important in the Mediterranean region due to its good adaptation to aquaculture conditions, its rapid growth, and the high market price of its salt-cured and dried eggs named bottarga (Whitfield et al., 2012).

The golden grey mullet, *Chelon auratus*, is currently another Mugilidae species of high economic value (Thomson, 1997; Ghelichi et al., 2004). The distribution of this species includes the coasts of the Mediterranean and

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the Black Sea, along the Atlantic coast from Senegal to the southern coasts of Norway and Sweden, the British Isles (but not the Baltic Sea), and the Caspian Sea. Among the abovementioned species, *C. auratus* is distributed in the Black Sea region of Turkey (Özer and Kirca, 2013).

Most of the research on *Chelon auratus* was performed in the central and eastern Mediterranean, particularly along all Aegean Sea coasts covering both the Greek and Turkish parts (Minos et al., 2002; Hotos, 2003; İlkyaz et al., 2006; Katselis et al., 2007, 2010). Previous studies have focused on different aspects of this species such as distribution and migration (Mickovic et al., 2010), age and growth (Mehanna, 2006; Fazli et al., 2008; Kraljevic et al., 2011), and reproduction (Hotos et al., 2000; Ghaninejad et al., 2010).

Fish, and poikilothermic animals in general, are influenced by environmental changes (Bastardo and Díaz-Barberán, 2005; Gabriel et al., 2004, 2007; Satheeshkumar et al., 2012a, 2012b). Moreover, various factors such as sex, age, reproductive cycle, feeding behavior, nutritional status, stress conditions, and water quality, in addition to the habitat of species, cause variations in blood parameters in fish (Leonardi and Klempau, 2003; Lim and Klesius, 2003; Cnaani et al., 2004). Therefore, it is necessary to examine them for a more precise evaluation of fish health status.

The present study aimed to research the hematological and biochemical parameters in Italian *M. cephalus* and Turkish *C. auratus* with close market prices according to global market indexes to compare the obtained results and to highlight the possible existence of significant differences in the hematological and biochemical profiles between these two species of mullet originating in two different habitats.

The results obtained in the present study will reveal further information on the adaptive responses of fish since the changes in blood parameters are strong biomarkers showing the environmental effects on fish physiology.

2. Materials and methods

2.1. Experimental design

The present study was performed on a total of 60 adult fish (30 flathead grey mullets, *M. cephalus*, and 30 golden grey mullets, *C. auratus*) caught in Italy and Turkey, respectively. Italian *M. cephalus* (271.00 ± 63.41 g weight, 33.58 ± 3.12 cm total length) were caught in three different stations at Lake Faro (Italy), while the Turkish *C. auratus* (273.80 ± 82.14 g weight, 34.46 ± 3.27 cm total length) were caught in three different stations in the Köyceğiz–Dalyan estuarine channel system (Turkey). Physical and chemical properties such as dissolved oxygen (DO), temperature, salinity, and pH of the water were determined in both sampling areas. In addition, all these parameters were determined by a multiparameter instrument in both Lake Faro and Köyceğiz–Dalyan (model YSI 556 MPS; YSI, Yellow Springs, OH, USA).

All fish were collected on the same day in February 2019 and were captured by seine. An external examination was carried out in all the fish samples for their general condition and health status and any marks of anomalies or infestation were investigated based on the assumption that all the samples were healthy. After being caught, all the fish samples were placed in a tank and anesthetized using 2-phenoxyethanol at 0.6 mg/L concentration.

2.2. Italian study area

Lake Faro is a hydrological formation that belongs to the coastal dune of Capo Peloro (38°15′58.86″N, 15°39′11.11″E) in the urban area of Messina, Sicily, Italy (Figure 1). Faro is a coastal lake and it has some characteristic structural properties like meromictic and strong layers. The lake is connected to the Ionian Sea by a channel system comprising two different layers, namely the H_2S layer in the hypolimnion and the red water layer at the chemocline (about 10 m). This red water layer was associated with phototrophic sulfur bacteria (Maugeri et al., 2000; Saccà et al., 2008).

2.3. Turkish study area

The Köyceğiz–Dalyan (36°49′15″N, 28°37′23″E) study area, in Muğla Province, is connected to the sea by a channel system. This connection is 14 km long and connects the Mediterranean Sea to Lake Köyceğiz, which has structural properties similar to those of Lake Faro, namely the ectogenic, crenogenic, and meromictic layers in the Köyceğiz–Dalyan Nature Reserve in the southwestern part of Turkey (Figure 2). The highest water depth value determined in the channel system is 4.5 m.

There are two different water layers, the mixohaline water of the mixolimnion of Lake Köyceğiz, which is the upper layer, and the saline water of the Mediterranean Sea and sulfuric thermal water sources, which is the lower layer comprising the water mass in the channel.

2.4. Blood sampling

Blood samples from a puncture of the caudal vein were taken immediately after anesthetization between 08:00 and 10:00 hours using a sterile plastic syringe (2.5 mL). The duration between fish capture and blood sampling was less than 5 min. Accordingly, two aliquots of each blood sample were taken into two different tubes (Miniplast 0.6 mL; LP Italiana Spa, Milan, Italy), one containing ethylenediaminetetraacetic acid (EDTA) (ratio: 1.26 mg/0.6 mL) as an anticoagulant agent for the determination of hematological properties (red blood cells, RBC; hemoglobin concentration, Hb; hematocrit, Hct; mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; and mean corpuscular hemoglobin concentration, MCHC) and the other containing no

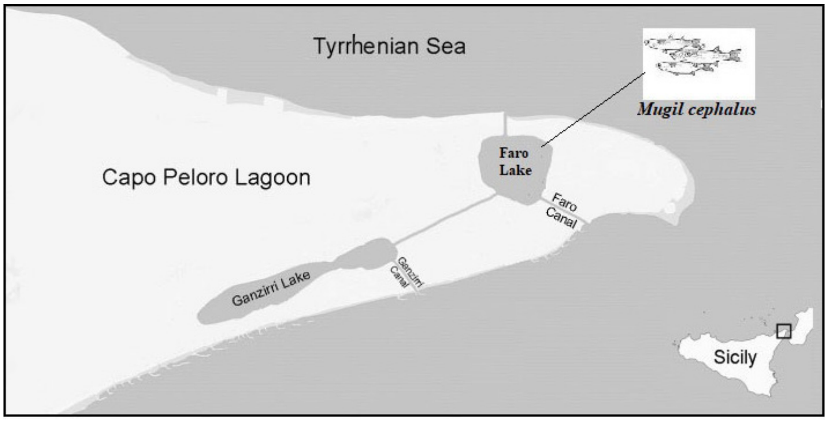

Figure 1. Map of the Italian study area. Location of Lake Faro in Sicily, Italy.

Figure 2. Map of the Turkish study area. Location of the Köyceğiz–Dalyan estuarine channel system in Turkey.

anticoagulant (after centrifugation) for the assessment of biochemical parameters (aspartate aminotransferase, AST; alanine aminotransferase, ALT; alkaline phosphatase, ALP; lactate dehydrogenase, LDH; serum total protein, TPROT; albumin, ALB; glucose, GLU; total cholesterol, CHOL; and triglycerides, TRIG).

Following the blood sampling procedure, the fish were weighed to ±0.1 g (balance Kern 440-49 N, Germany) and total length values were determined ±1.0 mm using an ichthyometer (Scubla SNC, 600 mm, Italy).

For the determination of the hematological parameters, RBC, Hct, and Hb were analyzed according to Blaxhall and Daisley (1973). RBC enumerations were carried out with a Neubauer hemocytometer (Shah and Altındağ, 2005), using Dacie's diluting fluid. The Hct value was analyzed using a capillary hematocrit tube. The Hb concentration was determined by spectrophotometric measurements at 540 nm according to the cyanmethemoglobin method. Erythrocyte index parameters like MCV, MCH, and MCHC values were determined indirectly by calculation using the directly measured parameters values according to the following formulae (Bain et al., 2006):

MCV = Hct × 10/RBC

MCH = Hg × 10/RBC

MCHC = Hb × 100/Hct

The blood was centrifuged at 740.34 × *g* for 4 min to separate the serum to be used for the biochemical determinations. The serum was then stored at –20 °C until further analysis. Biochemical parameters were determined using commercial kits (Giesse Diagnostics S.r.l., Rome, Italy) with an automated UV spectrophotometer analyzer (SEAC, Slim, Florence, Italy).

All the procedures adopted in the present study were carried out in compliance with the standards described by the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal experiments.

2.5. Statistical analysis

The values obtained were determined as mean ± standard deviation (SD) using three replicates.

Prior to the statistical analyses, all variables were checked for normality using the Kolmogorov–Smirnov test. Differences in the hematological and biochemical parameters between Italian (*M. cephalus*) and Turkish (*C. auratus*) mullet species were assessed by the unpaired t-test using a significance level of $P < 0.01$.

The statistical evaluations were performed by means of the statistical software Prism v. 7.00 (GraphPad Software Ltd., USA, 2003).

3. Results

Table 1 shows the mean values ±SD of biometric indices of Italian and Turkish mullets and water quality values of the two study areas (Lake Faro and the Köyceğiz–Dalyan estuarine channel system).

All water parameters recorded in the Italian study area were significantly higher than those of the Turkish study area. There were no significant differences between the biometric indices (weight and total length) of the two fish species.

Results for the evaluated hematological and biochemical parameters of all fish samples are given in Tables 2 and 3, respectively. The use of the unpaired t-test showed statistically significant differences ($P < 0.0001$) between the Italian and Turkish mullet species in all measured blood parameters, except for ALT. In particular, Hb, Hct, MCV, MCH, MCHC, AST, LDH, ALB, and TRIG were higher in Italian *M. cephalus* compared to Turkish *C. auratus*. In contrast, RBC, ALP, TPROT, GLU, and CHOL exhibited higher values in *C. auratus* compared to *M. cephalus*.

4. Discussion

The environmental and geographical variations in particular influence not only the hematological and biochemical parameters but also the biometric indices of fish (Fazio et al., 2012a, 2012b; Rahman et al., 2013; Boltaña, 2017). *M. cephalus* and *C. auratus* are the two most important species of the family Mugilidae for aquaculture. In addition, these two species are the fastest growing species of the family. In estuarine and pond habitats located in the Mediterranean basin, the average age-related lengths of these two species are very similar (Kara and Quignard, 2019).

In the present research the hematological profile and some biochemical parameters of Italian *M. cephalus* and Turkish *C. auratus* were evaluated. Except for ALT, the blood parameters measured exhibited significant differences ($P < 0.0001$) between the two mullet species. Previous studies showed the influence of biometric data such as weight and length on hematological parameters in several fish species (Adamu and Solomon, 2015; Fazio et al., 2017). However, although the blood parameters in the present study showed significant differences in fish sampled in Italy compared to those sampled in Turkey, the results related to biometric indices (weight and total length) showed no significant differences between the two mullet species.

These variations could represent an adaptive response of fish to the effects of the environment, clearly showing how the study of blood parameters could contribute to evaluations of the effect of habitat changes on fish and their health status.

Concerning the hematological profile, the results of the current study showed that Hb, Hct, MCV, MCH, and MCHC values were significantly higher in Italian *M. cephalus* than in Turkish *C. auratus*; however, RBC exhibited significantly lower values.

In the Italian study area, significantly higher values were recorded for water temperature, salinity, DO, and pH compared to the Turkish study area. In fact, a higher value of RBC could be expected due to the increased temperature linked to an increased metabolic rate and consequently higher oxygen consumption (Hazel and Prosser, 1974; Haschemeyer, 1978); however, this counterbalance was not seen in the present study, since RBC values for fish in the Turkish study area were higher despite the lower water temperature compared to the Italian study area.

The results in the present study showed a decrease in RBC, which was in line with the findings of an earlier study on rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792) (Fazio et al., 2016). Moreover, in a previous study, Solomon and Okomoda (2012) found higher values of RBC in fish exposed to an increasing number of light hours, showing an influence of photoperiod on blood parameters including RBC. It is very likely that this explanation can also support our results, and that the natural daylight cycle of the Turkish study area during the experimental period (April 2019) was characterized by more light hours compared to the Italian study area, possibly contributing to the higher values of RBC in *C. auratus* compared to those determined in *M. cephalus*.

Table 1. Water quality values (mean ± SD) for the two study areas in February 2019 and biometric data of Italian (*Mugil cephalus*) and Turkish (*Chelon auratus*) mullet species. Means without the same superscript letters within the same parameters are significantly different (P < 0.05).

Water parameters	Lake Faro, Italy	Köyceğiz–Dalyan estuarine channel, Turkey
Temperature (°C)	18.20 ± 0.09^{a}	15.63 ± 0.06^{b}
Water salinity (‰)	24.50 ± 0.17^{a}	3.08 ± 0.08^{b}
Dissolved oxygen (mg/L)	10.80 ± 0.15^{a}	7.28 ± 0.29^{b}
pH	8.97 ± 0.01^{a}	8.54 ± 0.25^{b}
Biometric parameters		
Weight (g)	271.00 ± 63.41^{a}	273.80 ± 82.14^{a}
Total length (cm)	33.58 ± 3.12^{a}	34.46 ± 3.27^{a}

Table 2. Statistical results for the evaluated hematological parameters in Italian (*Mugil cephalus*) and Turkish (*Chelon auratus*) mullet species from Lake Faro and the Köyceğiz–Dalyan estuarine channel, respectively. Means without the same superscript letters within the same parameters are significantly different (P < 0.05).

Parameters	Study areas	Mean ± SD	Median	95% confidence interval	25th–75th percentile range
RBC (×10^{6}/μL)	Italy	3.31 ± 0.33^{a}	3.32	2.67 ± 3.89	3.07 ± 3.59
	Turkey	5.05 ± 0.38^{b}	5.09	4.33 ± 5.91	4.82 ± 5.22
Hb (g/dL)	Italy	12.01 ± 1.23^{a}	12.00	10.51± 14.54	11.00 ± 12.70
	Turkey	6.46 ± 0.78^{b}	6.35	5.05 ± 7.64	5.99 ± 7.17
Hct (%)	Italy	37.25 ± 3.81^{a}	37.35	27.81 ± 43.06	35.73 ± 40.00
	Turkey	29.63 ± 1.13^{b}	30.00	27.55 ± 31.45	29.00 ± 30.25
MCV (fL)	Italy	113.2 ± 13.32^{a}	109.20	108.20 ± 118.10	103.10 ± 121.80
	Turkey	59.11 ± 5.18^{b}	58.46	57.17 ± 64.04	55.62 ± 62.41
MCH (pg)	Italy	36.45 ± 3.72^{a}	36.00	35.06 ± 37.84	33.83 ± 40.00
	Turkey	12.91 ± 2.06^{b}	12.56	12.14 ± 13.68	11.44 ± 14.22
MCHC (%)	Italy	32.53 ± 4.37^{a}	31.97	30.89 ± 34.16	28.86 ± 35.70
	Turkey	21.84 ± 2.83^{b}	21.54	20.78 ± 22.89	20.46 ± 24.11

RBC: Red blood cells; Hb: hemoglobin concentration; Hct: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

Biochemical differences in fish blood might be a result of changes in the physicochemical parameters of habitats (Fazio et al., 2013). The serum hepatic enzyme values in fish have been reported to vary depending on stress, tissue damage, and feeding habits in general. However, some studies have reported that some serum enzyme values vary depending on environmental factors (Shi et al., 2006). ALT and AST transferase enzymes are largely located in the liver and they are responsible for the amino acid metabolism in teleost fish. Serum transferase enzymes in fish varied depending on salinity (Fazio et al., 2013). Studies about the effect of salinity on fish serum transferase enzymes presented results similar to those obtained in the present study. Al-Khashali et al. (2013) reported that an increase in salinity significantly increased the serum AST value in teleosts, whereas there were only slight increases in serum ALT levels. Serum glucose level is a useful parameter to understand the response to environmental stress in fish (Jentoft et al., 2005). However, parameters that increase serum glucose level are not limited to stress. Numerous studies have reported an inverse relationship between glucose levels and temperature (White and Fletcher, 1985). Bayir et al. (2007) reported that serum glucose level increased in *Capoeta capoeta umbla*, a teleost, as a result of the decrease in water temperatures during winter. The changes in total protein levels in serum may have resulted

Table 3. Statistical results for the evaluated biochemical parameters in Italian (*Mugil cephalus*) and Turkish (*Chelon auratus*) mullet species from Lake Faro and the Köyceğiz–Dalyan estuarine channel, respectively. Means without the same superscript letters within the same parameters are significantly different (P < 0.05).

Parameters	Study areas	Mean ± SD	Median	95% confidence interval	25th–75th percentile range
AST (U/L)	Italy	38.4 3± 6.02[a]	38.00	36.19 ± 40.68	33.00 ± 44.00
	Turkey	23.38 ± 7.35[b]	22.39	20.64 ± 26.13	18.66 ± 26.56
ALT (U/L)	Italy	6.96 ± 0.89[a]	7.00	6.63 ± 7.29	6.48 ± 7.53
	Turkey	7.09 ± 2.74[a]	7.27	6.07 ± 8.11	4.69 ± 8.55
ALP (U/L)	Italy	19.30 ± 4.89[a]	20.00	17.48 ± 21.12	15.00 ± 22.25
	Turkey	217.20 ± 63.41[b]	205.70	193.50 ± 240.90	185.80 ± 252.40
LDH (U/L)	Italy	38.83 ± 8.30[a]	38.00	35.73 ± 41.93	32.75 ± 45.25
	Turkey	18.12 ± 6.10[b]	16.47	15.84 ± 20.40	13.83 ± 20.58
TPROT (g/L)	Italy	29.77 ± 3.95[a]	29.00	28.29 ± 31.24	26.75 ± 33.00
	Turkey	114.70 ± 24.24[b]	113.00	107.10 ± 122.20	99.26 ± 129.30
ALB (g/L)	Italy	12.60 ± 2.33[a]	12.00	11.73 ± 13.47	11.00 ± 14.00
	Turkey	1.76 ± 0.61[b]	1.50	1.53 ± 1.98	1.38 ± 2.02
GLU (mmol/L)	Italy	3.82 ± 0.45[a]	3.77	3.65 ± 3.99	3.52 ± 4.19
	Turkey	5.10 ± 1.39[b]	4.87	4.58 ± 5.62	3.94 ± 5.81
CHOL (mmol/L)	Italy	7.61 ± 0.51[a]	7.51	7.42 ± 7.80	7.31 ± 8.03
	Turkey	11.88 ± 2.12[b]	12.02	11.09 ± 12.67	10.45 ± 13.37
TRIG (mmol/L)	Italy	1.10 ± 0.14[a]	1.11	1.04 ± 1.15	1.02 ± 1.16
	Turkey	0.69 ± 0.17[b]	0.65	0.62 ± 0.75	0.57 ± 0.74

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; TPROT: serum total protein; ALB: albumin; GLU: glucose; CHOL: total cholesterol; TRIG: triglycerides.

from the structural adaptation of the liver. Changes in liver deamination capacity during adaptation to environmental conditions are directly proportional to aminotransferase activity. Kavadias et al. (2003) have reported that an increase in serum total protein levels may be due to a decrease in aminotransferase enzymes in teleost fish. Similar results were obtained in the present study. The serum AST levels in the sampled fish in Italy were lower compared to those of fish sampled in Turkey; however, serum total protein levels showed opposite correlations. Cholesterol and triglycerides are the fundamental lipids that create lipoproteins by bonding with proteins in the blood. The blood cholesterol concentration may vary depending on feeding amount and the preference of the fish (Aras et al., 2008). In the present study conducted in different habitats, it is thought that the difference in cholesterol levels of the mullet species was caused by feeding on different natural diets. Metabolism rate and serum cortisol and glucose levels were correlated with each other. The rate of metabolism is accelerated with the secretion of cortisol. As a result of this, the blood glucose level increases (Barton, 2002). Changes in environmental factors, especially changes in water parameters, increase cortisol levels, metabolic rate, and serum glucose levels in fish (Van Der Boon et al., 1991). Lidman et al. (1979) investigated the changes in serum triglyceride levels related to increasing metabolism rate with cortisol injection in the European eel. They reported that after injection triglyceride levels decreased, whereas serum glucose level increased with accelerated metabolism. A similar correlation between serum triglyceride and glucose level was found in the present study. Serum glucose levels in the mullets sampled in Turkey were high whereas triglyceride levels were low.

The present study contributes to the research on hematological and blood biochemical differences in two species with equal biometric profiles belonging to the family Mugilidae in distinct habitats and on fish response to environmental conditions. The obtained results expand the knowledge on the influence of different geographical locations on fish physiology. Further research is encouraged to expand the studies to other species present in the investigated areas.

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