Effect of two different habitat on haematological parameters and serum protein profile in *Mugil cephalus*

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Abstract. The aim of this study was to assess the influence of different water quality of Faro Lake and Tyrrhenian Sea on the haematological and serum proteins profiles of *Mugil cephalus*. In both sampling sites water physical-chemical-parameters of the water colum were measured. Our results showed statistical difference in pH, salinity and dissolved oxygen between the two sites. White blood cells, total proteins, prealbumin, albumin and α-globulins showed a statistical significance in mullet dwell in two different habitat (Faro lake and Tyrrhenian Sea). The variations in haematological parameters and serum protein profile are a good indicators to detect intra and inter-specific variations in relation to different aquatic habitats. These results represent important haematological indices useful for monitoring the effect of environmental changes and physiological adaptive capacities of mullet.

Keywords: haematological parameters, protein profile, mullet, Faro Lake, Tyrrhenian Sea.

INTRODUCTION

The Mugilidae family includes 17 genera and 72 species in the world (Turan et al., 2011).

Mugil cephalus is the most abundant among the mullet species with worldwide economic importance and worldwide distribution in topical, subtropical and temperate climates. This euryhaline marine teleost feeds mainly on zooplankton, benthic organisms and detritus.

M. cephalus have shown to be sufficiently sensitive to anthropogenic compounds in laboratory tests (Andrade et al. 2004) and therefore suitable for biomonitoring studies. In addition this species possesses several characteristics typical of estuarine sentinel species, such as the extreme salinity tolerance (Ferreira et al., 2005). Fish are known to be in close relationship with the water environment, hence, the blood will reveal conditions within the body of the fish long before there is any visible manifestation of disease (Musa and Omoregie, 1999; Okechukwu et al., 2007); haematological indices are therefore widely used by fish biologists and researchers the world over as early warn signal of changes in the health status of the animal. Fernades and Mazon (2003) reported that fish blood are closely related to the animal response to either natural or artificial changes in its environment. The easily of determining some blood parameters is probably responsible for the rise in the use of haematology as a tool for testing of health condition in fish (De Pedro et al., 2005). Haematological studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. As well as haematological parameters, also the determination of protein content in the blood plasma is a good indicator to detect intra and inter-specific variation among species, identifying population, for the ecological stress, physiological homeostasis and aquatic pollution (Sharaf-Eldeen and Abdel-Hamid 2002). In last decades a wealth of literature has been accumulated on blood plasma protein fraction in different animals including fish. Indeed many reports are available on electrophoresis studies of serum fractions related to the .health status of fish (Deutsch and Goodloe 1945; Hongkun et al., 2008), but little is known about the use of protein electrophoresis in fish for the monitoring of different aquatic habitat (Kekic and dos Remedios 1999; Muthukmaravel et al., 2007; Osman et al., 2010).

The main objective of this present study was to compare the haematological parameters and protein profile of *M. cephalus* captured in two different habitats, Faro lake and Tyrrhenian Sea, with the aim to understand how the difference in chemical-physical parameters of water can influence haematological parameters.

MATERIAL AND METHODS

Study area

Capo Peloro is a brackish system located in the north-eastern corner of Sicily (38° 15' 57" N; 15° 37' 50" E). It consists of two basins, Ganzirri and Faro, communicating with the Tyrrhenian Sea by English channel and connected to each other by Margi channel (Mazzola et al. 2010). Owing to the marine input, underground springs and meteorological and climatic conditions, the lakes of Capo Peloro are characterised by large fluctuations in chemico-physical variables, especially salinity, temperature and, mainly in Faro Lake, dissolved oxygen (Bergamasco et al., 2005).

Faro is a small meromictic marine lake (~26 ha) and is characterised by the presence of H_2S in the hypolimnion and a brownish water layer at the chemocline (at about 10m depth) colonised by dense populations of phototrophic sulphur bacteria (Vanucci et al. 2005). It is a circular basin with a 500m diameter, and is deeper in its central part (~30 m), whereas its mean depth ranges from 0.5-5m. The lake is characterised by sandy-muddy bottoms seasonally covered by green algal mats, although primary production here is mainly sustained by phytoplankton (Manganaro et al., 2009).

Together with Ganzirri, the lake of Faro represents a site of ethno anthropological interest thanks to the the traditional manufacturing activities related to Shellfish breeding. In fact, Faro is largely exploited for bivalve cultivation (mainly *Mytilus galloprovincialis*). The bottom of Tyrrhenian Sea, in general in the Strait of Messina, degrades slowly reaching the 500 meters of depth between the two shores of Sicily and Calabria. The Tyrrhenian waters are strongly influenced by tidal exchange regime, typical of the Messina Strait (De Domenico 1987). In the area of sampling, the nature of the seabed is rocky. This is part of a coastal habitat of particular interest, consisted of a peculiar biocenotic complex. This is an extended stretch of coast from Cape Peloro to S. Agata, affected by the presence of a rocky bench, that from the shore line, goes up to several meters deep. This training, interpretable as a "beach rock", is located in a position of connection between the plane and the fringe mesolittoral upper sublittoral. This structure is the only natural hard substrate for benthic communities within this zone depth range, along the Sicilian side of the Strait.

Sampling and analytical methods

For our study 30 *M. cephalus* (Fig. 1) were investigated and they were divided into two equal groups on the basis of the site of collection. 15 fish were caught in Faro Lake and 15 were caught in Tyrrhenian Sea. All fish, caught with bottom-set nets, were transferred in different tanks and anaesthetized prior to blood sampling using 2-phenoxyethanol (99%, MERCK, Whitehouse Station, NJ, USA) at the concentration of 400 mg/l. At the end of blood sampling on all subjects weight and length were recorded (Faro Lake: length 31.53 ± 1.09, weight 416.50 ± 14.91; Tyrrhenian Sea: length 30.45 ± 1.03, weight 403.30 ± 16.14). On the basis of their weight and length all fish were considered sexually mature and with age between two and four years (McDonough et al., 2005). Withdrawal did not cause adverse effect or death on all subjects that returned to the wild.

For both sites of collection (Fig. 1) we measured chemical-physical parameters of the water. Water sampling was carried out in the same date of fish sampling, in three stations of Faro Lake and Tyrrhenian Sea. The three stations on the each location were selected randomly and the distances among them were about 3 meters.

Niskin bottle (General Oceanics, Inc.-Miami, Florida) for sampling and a multiparametric probe YSI 85 System for temperature, salinity, dissolved oxygen (DO) and pH.
Physical-chemical parameters did not present statistical differences among the three monitoring points at each site and showed a CV less than 18%.

Blood samples were collected by caudal vein 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^{-1}) as an anticoagulant agent and the other without EDTA. The blood samples collected in EDTA tubes were used for the determination of haematological profile. It was measured within 1 hour after blood sampling by an automated haematology analyzer (HeCo Vet C, SEAC, Florence, Italy) with special lysing reagent (SEAC) containing potassium cyanide, ammonium quaternary salts, surfactants (Fazio et al., 2012a).

Fig. 1 – Image of *Mugil cephalus* euryhaline marine teleost.

Evaluation of the haemogram involves the determination of the Red Blood Count (RBC), Haematocrit (Hct), Hemoglobin concentration (Hgb), White Blood Cell Count (WBC), Thrombocyte Count (TC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC). Total protein and electrophoretic profile were determined on serum samples obtained from blood samples without EDTA by centrifugation for 10 min at 3000 rpm.

The concentration of serum total proteins was determined by biuret method using an automated analyzer UV Spectrophotometer (SEAC, Slim, Florence, Italy). The protein fractions were performed using an automated system (Sel Vet 24, SELEO Enginering, Naples, Italy) according to the procedures described by the manufacturer. For each sample, 25 µl of serum were applied to numbered sample wells. Each holder accommodate up to 24 samples. Films were electrophoresis for about 30 minutes at 165V. After electrophoresis, films were simultaneously fixed using an automated system, stained in red stain acid solution for 3 minutes, and then dried at 37 °C.

After destaining in acetic acid and drying completely for about 10 minutes films were scanned on a densitometer, electrophoretic curves plus related quantitative specific protein concentrations for each sample were displayed. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentration (g/dl) were calculated using the total protein concentration.

Protocols of fish and experimentation were reviewed and approved in accordance with the standards recommended by the *Guide for the Care and Use of Laboratory Animals* and Directive 86/609 CEE.

Statistical Analysis

Data obtained for biometric data and different blood and serum parameters were tested for normality using Kolmogorov-Smirnov test. $P < 0.05$ was considered statistically significant. Unpaired T-test was used to determine significant differences in chemical and physical parameters of two sampling sites, between biometric data, haematological parameters and proteins profile measured in two groups. $P < 0.05$ was considered statistically significant. Data were analyzed at 95% confidence level and all calculations were carried out using statistical software Prism v. 4.00 (Graphpad Software Ldt., USA, 2003).

RESULTS

Physico-chemical parameters (mean±SD), measured in water at both sites, are represented in table 1. Unpaired T-test showed statistical differences in pH ($P = 0.0356$), salinity ($P = 0.0155$), dissolved oxygen ($P = 0.0499$) values between the two monitoring sites. No significant differences were found in length and weight between two groups. Unpaired T-test showed significantly lower levels of WBC ($P = 0.0115$), total proteins ($P = 0.0038$), prealbumin ($P < 0.0001$), albumin ($P < 0.0001$) and α-globulins ($P = 0.0009$) in mullet caught in Faro Lake respect to those in Tyrrhenian Sea (Fig. 2).

DISCUSSION

The physiological and biochemical characteristics of blood in fish are sensitive to changes in the environmental medium (Atamanalp et al., 2002) because fish are intimately associated

Tab. 1 – Chemical and physical parameters of waters investigated in the two sampling sites. **T** = Temperature; **Sal** = Salinity; **DO** = Dissolved Oxygen. Significance: * Vs Tyrrhenian Sea (P < 0.05)

Parameters	**Faro Lake**	**Tyrrhenian Sea**	**Percentage Change**
T (°C)	25.30 ± 0.51	22.90 ± 0.70	9.49 %
pH	8.11 ± 0.02*	8.20 ± 0.02	1.10 %
Sal (‰)	33.43 ± 0.81*	36.80 ± 0.15	9.16 %
DO (ml/l)	5.89 ± 0.06*	4.99 ± 0.31	15.30 %

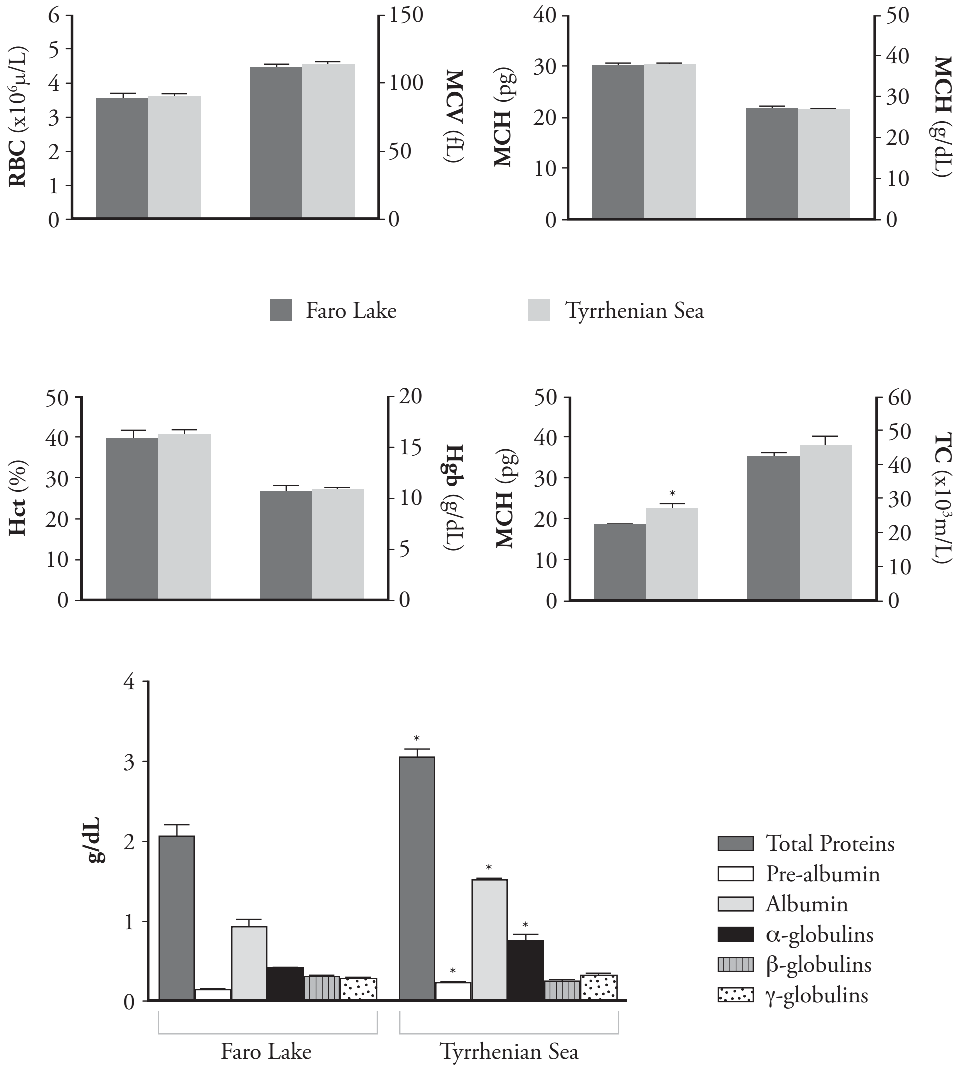

Fig. 2 – Mean value ± SD of parameters in *Mugil cephalus* captured in two different site (Faro Lake and Tyrrhenian Sea) with the statistical significances. * Significance Vs Faro Lake (P < 0.05)

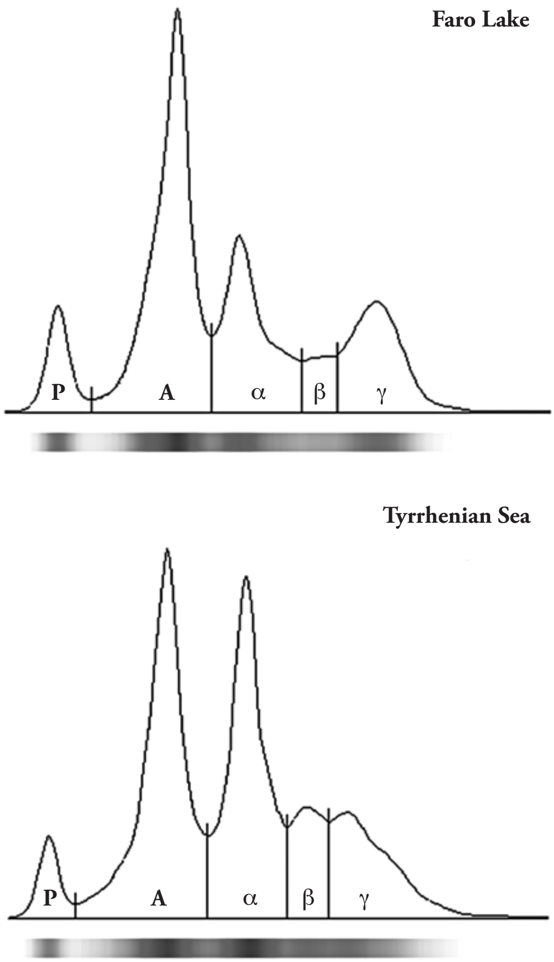

Fig. 3 – Five fractions obtained in serum, correlating to prealbumin (fraction I), albumin (fraction II), α-globulins, β-globulins, γ-globulins in in *Mugil cephalus* captured in two different site: Faro Lake and Tyrrhenian Sea.

with the water environment; so physical and chemical changes in the environment are reflected on physiological parameters of fish (Musa and Omoregie 1999). Haematological parameters such as total proteins can be useful as biomarkers of different aquatic habitat of fish (Maceda-Veiga et al., 2010). In our study pH, salinity, DO showed a significantly increase ranging between 1.10% and 15.30% in Faro Lake than in Tyrrhenian Sea. These variations, overall pH and salinity, could cause a physiological adaptive response on *M. cephalus* that moves from one habitat to the another. Previous researches showed that physico-chemical differences in the habitats influence the haematological parameters and serum biochemistry in fish suggesting that hematological parameters may be suitable for monitoring the effects of habitat changes on fish biology and fish culture practices (Fazio et al., 2012b, 2012c).

Fish exposed to chronic stress such as pH and salinity variation, manifest lymphopenia and, in some cases, monocytosis (Cazenave et al., 2009; Davis et al., 2008). Our data are in according to Jerônimo et al. (2009) that observed a lower WBC value in fish captured in different sites.

In *M. cephalus* five fractions were obtained in serum, correlating to prealbumin (fraction I), albumin (fraction II), α-globulins, β-globulins, γ-globulins. These fractions are shown in figure 3. Statistical analysis showed highest total proteins, prealbumin, albumin and α-globulins levels in mullet lived in Tyrrhenian Sea. This is in contrast with other studies reporting either no changes (Woo and Murat 1981) or decreases (Kelly and Woo 1999) in plasma protein levels in parallel with increased salinity. The possible importance of increased plasma protein as a fuel for tissues during osmotic acclimation has not been addressed yet but may be related to a metabolic reallocation of energy resources in hyperosmotic environments once carbohydrate stores have been mobilized.The reduction of serum protein concentration in mullet lived in Faro Lake can be due to the protein catabolism, the process converting blood and structural protein to energy, to meet the higher energy demand during the different values of pH (Das et al., 2006).

Moreover these results confirm that *M. cephalus* are able to adapt to a wide range of environmental salinities and pH were facing extra energy costs, probably related to osmoregulatory processes. Amino acids seem to play an important role so fish can adjust to the different environmental salinities, either as energy sources or as important osmolytes for cell volume regulation (Costas et al. 2012).

The variations in WBC and in protein profile due to different habitat emphasize the fact that changes in blood characteristics are important indices in monitoring the effect of environmental changes and adaptive capacities of fish physiology.

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RIASSUNTO

Effetto di due differenti habitat sui parametri ematologici e sul profilo elettroforetico nel Cefalo *Mugil cephalus*

La famiglia dei Mugilidi include 17 generi e 72 specie la cui distribuzione è circumtropicale, cioè relativa alla presenza di acque tropicali e temperate calde. Il Mugil cephalus è una specie eurialina, ossia in grado di tollerare ampie variazioni di salinità tanto da riuscire a vivere in acque marine, dolci e salmastre. È una specie particolarmente resistente capace di adattarsi anche ad ambienti inquinati. Vista la capacità adattativa di tale specie, in tale studio sono stati valutati i parametri ematologici e il profilo elettroforetico di cefali appartenenti a due habitat differenti: il lago Faro, un piccolo pantano meromittico marino, situato nella punta nord-est della Sicilia vicino al promontorio di Capo Peloro (38°15'57" N; 15° 37' 50" E) e il Mar Tirreno. Prima di procedere con la sperimentazione, nei siti di cattura sono stati effettuati campionamenti di acqua al fine di valutarne i principali parametri chimico-fisici (temperatura, pH, salinità e ossigeno disciolto) mediante l'utilizzo di una sonda multiparametrica (YSI 85 System). Nel nostro studio sono stati utilizzati in tutto 30 cefali: 15 catturati nel lago di Faro e 15 nel Mar Tirreno. Su tutti gli esemplari, anestetizzati con 2-fenossietanolo (99%, MERCK,

Whitehouse Station, NJ, USA), è stato effettuato il prelievo di sangue dalla vena caudale. I campioni di sangue sono stati suddivisi in due aliquote in due differenti provette: Miniplast 0.5 ml (LP Italiana Spa, Milano) contenente EDTA (1.26 mg /0.5 ml) come agente anticoagulante, provette prive di anticoagulante. Su sangue intero, ottenuto dalle provette contenenti EDTA, sono stati valutati alcuni parametri ematologici quali il conteggio dei globuli rossi (RBC), ematocrito (Hct), emoglobina (Hb), conteggio dei globuli bianchi (WBC), conteggio dei trombociti (TC), volume cellulare medio (MCV), contenuto cellulare medio dell'emoglobina (MCH), concentrazione corpuscolare media emoglobinica (MCHC) utilizzando un contaglobuli elettronico (HeCo Vet C, SEAC, Firenze). Sui sieri, ottenuti mediante centrifugazione (300rpm x 10min) dalle provette prive di anticoagulante, sono stati valutati le concentrazioni delle proteine totali attraverso spettrofotometria UV (SLIM, SEAC Firenze) e il profilo elettroforetico mediante un sistema automatico (Sel Vet 24, SELEO Enginering, Napoli). I risultati ottenuti hanno mostrato una diminuzione statisticamente significativa delle proteine totali, della prealbumina, dell'albumina e delle α-globuline nei cefali catturati nel lago rispetto a quelli pescati nel Mar Tirreno. Queste variazioni potrebbero essere attribuite al diverso ambiente acquatico le cui caratteristiche chimico-fisiche hanno evidenziato variazioni significative. In particolare, la diminuzione del pH e della salinità riscontrate nel lago di Faro potrebbero essere responsabili di un esaltato catabolismo proteico che, utilizzato a scopo energetico, ridurrebbe in modo significativo la componente proteica dei soggetti. Allo stesso modo i più bassi livelli di WBC ritrovati nei cefali di lago sembrano essere la "risposta ematologica" di questa specie alle differenti condizioni chimico-fisiche dell'ambiente acquatico. Le stesse variazioni sono già state riscontrate in altre specie da diversi autori (Cazenave et al., 2009; Jerônimo et al., 2009; Davis et al., 2008). I risultati da noi ottenuti mettono in luce l'importanza di monitorare i parametri ematologici e il profilo proteico in pesci che vivono in differenti habitat, enfatizzando per alcuni di essi, il ruolo di bioindicatori utili alla valutazione non solo lo stato di salute degli animali ma anche al monitoraggio dell'ecosistema e dell'ambiente.

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