Ichthyol Res (2013) 60:36–42
DOI 10.1007/s10228-012-0303-1

FULL PAPER

Haematological and serum protein profiles of *Mugil cephalus*: effect of two different habitats

Francesco Fazio · Simona Marafioti · Agata Torre · Marilena Sanfilippo · Michele Panzera · Caterina Faggio

Received: 15 February 2012 / Revised: 11 July 2012 / Accepted: 11 July 2012 / Published online: 21 August 2012

Abstract The aim of this study was to assess the influence of two different habitats, Faro Lake (group A) and Tyrrhenian Sea (group B), on the haematological and serum protein profiles of *Mugil cephalus*. Our results showed significant differences of white blood cells, total proteins, prealbumin, albumin and α-globulins between groups A and B. These findings suggest that changes in haematological and serum protein profiles are important indices in monitoring the effects of aquatic habitat changes, representing an adaptive physiological response to different habitats of *M. cephalus*.

Keywords Blood parameters · Electrophoretic patterns · Mullet · Water environment

Introduction

Fish, as bioindicator species, play an important role in monitoring of water quality because they respond with great sensitivity to changes in the aquatic environment (Borkovic et al. 2008). Fish can be affected directly or indirectly. The direct effects concern the lower level of biological organization; indirect effects concern the food chain and the behaviour of the organism (Osman et al. 2007a, b).

Physical and chemical changes in the aqueous environment often cause various physiological changes in fish; thus, the water quality of an aquatic habitat is crucial, because it determines the productivity and other parameters necessary for fish survival. Biomarker analysis of field-collected organisms can provide information on the status of the environment, avoiding the need for and uncertainty inherent to extrapolation of laboratory results (Menezes et al. 2006). Biomarkers are defined as a change in a biological response, ranging from molecular to cellular and from physiological responses to behavioural changes, which can be related to change of the aquatic habitat (Depledge et al. 1995). Use of selected biomarkers has become attractive and useful for monitoring environmental quality and the health of fish inhabiting polluted ecosystems (Fernandes et al. 2008). The easy determination of some blood parameters is probably responsible for the rise in the use of haematology as a tool for testing of health problems in fish (De Pedro et al. 2005). Haematological parameters of fish are closely related to their response to environmental and biological factors (Fernandes and Mazon 2003). As well as haematological parameters, also the determination of protein content in blood plasma is a good indicator to detect intra- and inter-specific variation among species, identify populations, and for investigation of ecological stress, physiological homeostasis and aquatic pollution (Sharaf-Eldeen and Abdel-Hamid 2002). Serum proteins are very complex and are involved in a wide range of physiological functions in both healthy and disease

F. Fazio · S. Marafioti (✉) · M. Panzera
Department of Experimental Science and Applied Biotechnology, Faculty of Veterinary Medicine, Polo Universitario Annunziata, University of Messina, 98168 Messina, Italy
e-mail: smarafioti@unime.it

A. Torre · C. Faggio
Department of Life Science "M. Malpighi" Section of General Physiology, Faculty of Science MM.FF.NN., University of Messina, Viale Ferdinando Stagno D'Alcontres 31, S. Agata, 98166 Messina, Italy

M. Sanfilippo
Department of Animal Biology and Marine Ecology, Faculty of Science MM.FF.NN., University of Messina, Viale Ferdinando Stagno D'Alcontres 31, S. Agata, 98166 Messina, Italy

states, which is why they play a role of great importance for zoologists, enzymologists, immunologists and toxicologists. In the last decade a wealth of literature has been accumulated on blood plasma protein fraction in different animals, including fish. Indeed many reports on electrophoresis studies of serum fractions from healthy fish have been published (Deutsch and Goodloe 1945; Hongkun et al. 2008), but little is known about use of protein electrophoresis in fish for monitoring of different aquatic habitats (Kekic and dos Remedios 1999; Muthukmaravel et al. 2007; Osman et al. 2010).

In this study, we used mullet as a sentinel organism, because this fish has been shown to be sufficiently sensitive to anthropogenic compounds in laboratory tests (Andrade et al. 2004) and therefore suitable for biomonitoring studies. Mullet (*Mugil cephalus*) is a perciform species that feeds mainly on zooplankton, benthic organisms and detritus, being chosen because it possesses several characteristics required in an estuarine sentinel species, such as extreme salinity tolerance (Ferreira et al. 2005). The purpose of this study is to evaluate variations of haematological parameters and electrophoretic pattern in *M. cephalus* captured at two different sites in response to changes of aquatic habitat.

Materials 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 the English Channel and with each other by the 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 (about 26 ha) and is characterised by the presence of H_2S in the hypolimnion and a brownish water layer at the chemocline (at about 10 m depth) colonised by dense populations of phototrophic sulphur bacteria (Vanucci et al. 2005). It is a circular basin with 500 m diameter, and is deeper in its central part (about 30 m), whereas its mean depth ranges from 0.5 to 5 m. 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, Faro Lake was declared of ethno-anthropological interest, being particularly important as the historical seat of 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, slopes slowly, reaching 500 m depth between the two shores of Sicily and Calabria. The Tyrrhenian waters are strongly influenced by a 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, consisting 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, extending from the shoreline to several metres deep. This feature, 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 the zone of this depth range, along the Sicilian side of the strait.

Sampling and analytical methods. For our study, 30 *Mugil cephalus* were investigated in May 2010. They were divided into two equal groups on the basis of site of collection. Fifteen fish were caught in Faro Lake (group A), and 15 were caught in Tyrrhenian Sea (group B). All fish were caught with bottom-set nets and immediately transferred to a tank. The fish were anaesthetized prior to blood sampling using 2-phenoxyethanol (99 %; Merck, Whitehouse Station, NJ, USA) at concentration of 400 mg/l. At the end of blood sampling on all subjects, 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 2 and 4 years (McDonough et al. 2005). Only male fish were used in this study. All animals were returned to the wild.

Table 1 Descriptive statistics of biometric data in 30 *Mugil cephalus* taken from Faro Lake and Tyrrhenian Sea

Collection site	Biometric parameters							
	Length (cm)				Weight (g)			
	Mean ± SEM	Min	Max	CV (%)	Mean ± SEM	Min	Max	CV (%)
Faro Lake ($n = 15$)	31.53 ± 1.09	20.00	38.00	13.39	416.50 ± 14.91	300	530	13.86
Tyrrhenian Sea ($n = 15$)	30.45 ± 1.03	17.50	34.50	13.06	403.30 ± 16.14	240	510	15.50

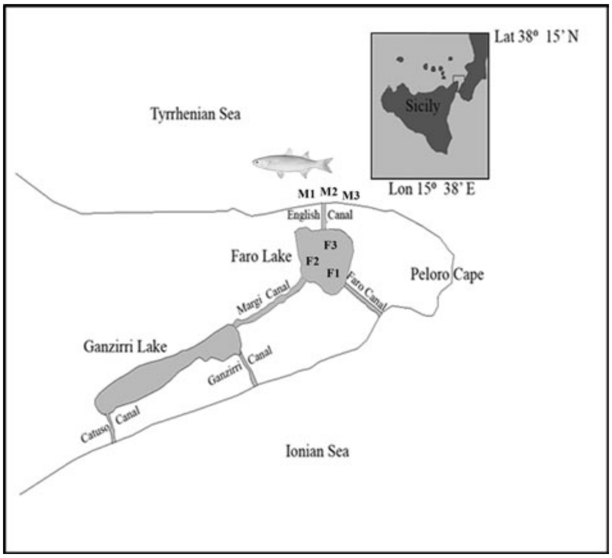

Fig. 1 Map of the study sites

For both sites of collection (Fig. 1) we measured chemical and physical parameters of the water. Water sampling was carried out on the same date as fish sampling, at three stations of Faro Lake (F1, F2 and F3) and Tyrrhenian Sea (M1, M2 and M3). The three stations at each location were selected randomly, and the distances among them were about 3 m.

Niskin bottle (General Oceanics, Inc., Miami, FL, USA) for sampling and a multiparametric probe YSI 85 system for temperature, salinity, dissolved oxygen (DO) and pH were used. Water samples were screened through a 200-µm-mesh net to remove large zooplankton and debris. Sub-samples (500–2000 ml) were filtered onto pre-washed, precombusted (450 °C, 4 h) and preweighed Whatman GF/F filters (0.45 µm nominal pore size). Filters were analysed for total suspended matter (TSM, mg/l), its inorganic (SIM, mg/l) and organic fractions (SOM, mg/l), and chlorophyll *a* (CHL*a*, µg/l). For determination of TSM, Whatman GF/F filters (0.45 µm nominal pore size) were weighed after desiccation (60 °C, 24 h) using a Mettler M3 balance (accuracy ±1 µg), while SOM was determined by loss on ignition (450 °C, 4 h; Strikland and Parsons 1992). Samples for dissolved oxygen analysis were also taken to carry out laboratory analysis with the Winkler method for testing the probe (Strikland and Parsons 1992). Samples for CHL*a* were treated and analysed according to Innamorati et al. (1990), using a Shimadzu UV-1800 UV/visible spectrophotometer. Physical–chemical parameters did not present statistical differences among the three monitoring points at each site and showed CV less than 18 %.

Blood samples were collected from 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 ethylenediamine tetraacetic acid (EDTA, 1.26 mg/0.6 ml) as an anticoagulant agent, and the other without EDTA. The blood samples collected in EDTA tubes were used for determination of the haematological profile, which was measured within 1 h after blood samples were taken using an automated haematology analyzer (HeCo Vet C; SEAC, Florence, Italy) with special lysing reagent (SEAC) containing potassium cyanide, ammonium quaternary salts and surfactants. Evaluation of the haemogram involves determination of the red blood count (RBC), haematocrit (Hct), haemoglobin concentration (Hgb), white blood cell count (WBC), thrombocyte count (TC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Using serum samples obtained from blood samples without EDTA subjected to centrifugation for 10 min at 3000 rpm, total protein and electrophoretic profile were determined. The concentration of serum total proteins was determined by biuret method using an automated UV spectrophotometer (SEAC; Slim, Florence, Italy). The protein fractions were determined using an automated system (Sel Vet 24; SELEO Engineering, Naples, Italy) according to the procedures described by the manufacturer. For each sample, 25 µl serum was applied to numbered sample wells. Each holder accommodates up to 24 samples. Films were electrophoresed for about 30 min at 165 V. After electrophoresis, films were simultaneously fixed using an automated system, stained in red stain acid solution for 3 min and then dried at 37 °C. After destaining in acetic acid and drying completely for about 10 min, films were scanned using a densitometer, and 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 concentrations (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 protein profiles measured in group A and group B. $P < 0.05$ was considered statistically significant. Data were analyzed at 95 % confidence level, and all calculations were carried out using Prism version 4.00 statistical software (GraphPad Software Inc., USA, 2003).

Table 2 Chemical and physical parameters of waters investigated at the two sampling sites

Parameter	Unit	Faro Lake (group A)				Tyrrhenian Sea (group B)				Percentage change
		F1	F2	F3	Mean ± SEM	M1	M2	M3	Mean ± SEM	
T	°C	26.00	25.60	24.30	25.30 ± 0.51	22.30	22.10	24.30	22.90 ± 0.70	9.49
pH		8.13	8.07	8.13	8.11 ± 0.02*	8.21	8.16	8.23	8.20 ± 0.02	1.10
Sal	‰	34.30	31.80	34.20	33.43 ± 0.81*	36.70	36.60	37.10	36.80 ± 0.15	9.16
DO	ml/l	5.91	5.99	5.78	5.89 ± 0.06*	4.50	4.88	5.59	4.99 ± 0.31	15.30
O_2	sat %	126.22	125.17	119.69	123.70 ± 2.02*	102.36	101.30	103.48	102.38 ± 0.62	17.23
TSM	mg/l	8.57	6.29	4.40	6.42 ± 1.20	4.68	4.43	4.39	4.50 ± 0.09	29.91
SIM	mg/l	4.86	3.71	1.60	3.39 ± 0.95	3.46	3.30	2.85	3.20 ± 0.17	5.61
SOM	mg/l	3.71	2.57	2.80	3.02 ± 0.34*	1.22	1.13	1.54	1.30 ± 0.05	56.95
CHL*a*	μg/l	5.32	3.51	4.35	4.39 ± 0.52*	0.42	0.55	0.35	0.44 ± 0.05	89.98

T temperature, *Sal* salinity, *DO* dissolved oxygen, *TSM* total suspended matter, *SIM* suspended inorganic matter, *SOM* suspended organic matter, *CHLa* chlorophyll *a*

* Significance versus group B: $P < 0.05$

Results

The chemical and physical features of the two monitoring sites (Faro Lake and Tyrrhenian Sea) are presented in Table 2. Unpaired *t* test showed statistical differences in some chemical and physical parameters measured in Faro Lake and Tyrrhenian Sea. Statistical differences were found in pH ($P = 0.0356$), salinity ($P = 0.0155$), dissolved oxygen ($P = 0.0499$), oxygen saturation ($P = 0.0006$), SOM ($P = 0.0080$) and CHL*a* ($P = 0.0017$) values. In particular, pH and salinity were higher in Tyrrhenian Sea with respect to Faro Lake with a difference of 0.09 and 3.37 ‰, respectively. On the contrary, DO, oxygen saturation, SOM and CHL*a* resulted higher in Faro Lake than in Tyrrhenian Sea. At the two monitoring sites, DO showed a difference of about 0.90 ml/l and oxygen saturation showed a difference of about 21.32 sat %. These high dissimilarities between the two monitoring sites were due to SOM, which in the lake showed a value more than twice that in the sea, and CHL*a*, which in the lake assumed a value ten times greater than in the sea.

No significant differences were found in length and weight between the two groups of fish. Among the haematological parameters considered (Table 3), unpaired *t* test showed statistical differences only in WBC ($P = 0.0115$). In particular, WBC value was lower in Faro Lake with respect to Tyrrhenian Sea with a difference of 3.99×10^3/μl (Table 3). In addition, in the present research, different patterns of serum proteins of *Mugil cephalus* were identified. In particular, five fractions were obtained in the serum, correlating to prealbumin (fraction I), albumin (fraction II), α-globulins, β-globulins and γ-globulins (Fig. 2). For protein profiles, unpaired *t* test showed significantly lower levels of total proteins ($P = 0.0038$), prealbumin ($P < 0.0001$), albumin ($P < 0.0001$) and α-globulins ($P = 0.0009$) in group A with respect to group B (Table 4).

Discussion

Environmental risk assessment of waters has traditionally been based on measurement of chemical and physical parameters of water and has included biological quality elements. Fish are intimately associated with the aqueous environment; physical and chemical changes in the environment are rapid and reflected as measurable physiological changes in fish (Musa and Omoregie 1999). These changes include not only blood parameters but also fish reproduction. As shown by Ferreira et al. (2011), spermatogenesis and gonad development in *Lipophrys pholis* were influenced by aquatic pollution. The physiological and biochemical characteristics of fish blood are easily modified by environmental changes (Atamanalp et al. 2002). Haematological parameters such as total proteins can be useful as biomarkers of different aquatic habitats of fish (Maceda-Veiga et al. 2010).

Our results showed some significant changes of physical and chemical parameters at the two monitoring sites studied (Table 2). SOM and CHL*a* showed significant changes in the lake with respect to the sea, with an increase of concentration, respectively, of 56.95 and 89.98 %. In particular, the concentrations of CHL*a* (on average about 4.39 μg/l) were significantly (10 times) higher in Faro Lake than Tyrrhenian Sea. Nevertheless, concentrations measured at Faro Lake were negligible with respect to the threshold value needed to avoid eutrophication recommended for northern European waters (CHL*a* 10 μg/l; CSTT, 1994). That is probably due to an alteration of the

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

Haematological parameters	Faro Lake (group A)			Tyrrhenian Sea (group B)		
	Min	Max	Mean ± SEM	Min	Max	Mean ± SEM
RBC ($\times 10^6$/µl)	2.21	4.47	3.53 ± 0.16	2.93	4.37	3.59 ± 0.09
Hct (%)	21.00	50.00	39.60 ± 2.10	30.80	46.40	40.59 ± 1.088
Hgb (g/dl)	5.70	13.30	10.65 ± 0.60	8.90	12.70	10.79 ± 0.25
WBC ($\times 10^3$/µl)	16.30	21.00	18.30 ± 0.40*	13.40	30.90	22.29 ± 1.34
TC ($\times 10^3$/µl)	35.00	51.00	42.00 ± 1.20	29.00	74.00	45.23 ± 2.89
MCV (fl)	96.00	137.00	111.40 ± 2.34	103.20	128.00	113.30 ± 2.06
MCH (pg)	25.70	34.50	29.89 ± 0.60	27.11	32.94	30.10 ± 0.41
MCHC (g/dl)	21.20	31.80	26.93 ± 0.62	23.20	28.90	26.65 ± 0.37

* Significance versus group B: $P < 0.05$

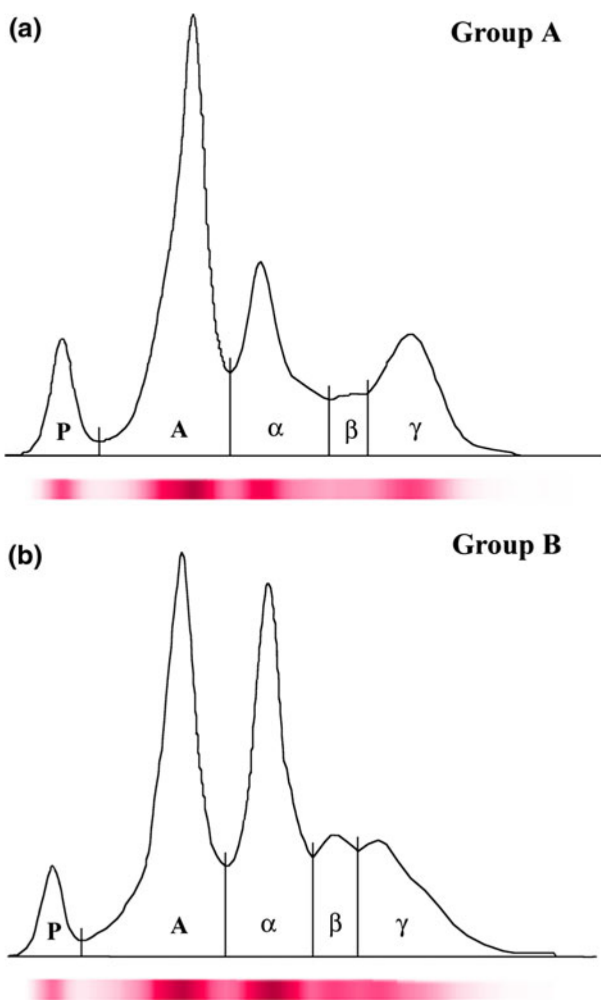

Fig. 2 Representative serum protein electrophoretograms observed in *Mugil cephalus* from two different sites. **a** Faro Lake, **b** Tyrrhenian Sea. Different patterns were identified as follows: *P* prealbumin, *A* albumin, *α* α-globulins, *β* β-globulins, *γ* γ-globulins

Table 4 Mean ± SEM values of total proteins and their fractions obtained in the two experimental groups

Parameter (g/dl)	Faro Lake (group A), mean ± SEM	Tyrrhenian Sea (group B), mean ± SEM	*P* value
Total proteins	2.05 ± 0.16*	3.05 ± 0.10	<0.0001
Pre-albumin	0.14 ± 0.02*	0.23 ± 0.02	0.0038
Albumin	0.92 ± 0.11*	1.51 ± 0.05	<0.0001
α-Globulins	0.41 ± 0.02*	0.75 ± 0.09	0.0009
β-Globulins	0.30 ± 0.03	0.25 ± 0.02	0.2217
γ-Globulins	0.27 ± 0.03	0.32 ± 0.03	0.2777

* Significance versus group B: $P < 0.05$

nitrogen pool, as surplus nitrogen due to waste discharge could easily be metabolized by bacteria and phytoplankton (La Rosa et al. 2002). pH, salinity, DO and oxygen saturated showed significant changes ranging between 1.10 and 17.23 % in Faro Lake with respect to Tyrrhenian Sea. These variations in overall pH and salinity could cause a physiological adaptive response on *Mugil cephalus* that moves it from one habitat to another.

Fish exposed to chronic stress (e.g. pH and salinity variation, contamination, infectious agents, predation) manifest lymphopaenia and, in some cases, monocytosis (Cazenave et al. 2009; Davis et al. 2008). In *M. cephalus*, the haematologic response to changes in aquatic habitat affected only the number of white blood cells, which showed a significant decrease in Faro Lake compared with Tyrrhenian Sea (Fig. 1). White blood cells play a major role in the fish defence system, and the percentage of each leucocyte type is a valuable tool for assessing fish condition (Cazenave et al. 2009; Houston 1997). Our data are in accordance with those of Jerônimo et al. (2009), who observed a lower WBC value in fish captured in different polluted sites. In our study, the water quality of Faro Lake is lower than in Tyrrhenian Sea, due to higher levels of SOM and CHL*a*. It is believed that SOM itself does not damage fish, but the decomposition action of organic matter by bacteria contributes to greater water turbidity that causes stress (Wahbi et al. 2004), manifested in this case by decrease in WBC. It is known that white blood cells respond to various stressors including infections and chemical irritants (Christensen et al. 1978) and different physical and chemical changes in aqueous environment. Thus increasing or decreasing numbers of white blood cells are a normal reaction on exposure to toxicants (Kori-Siakpere et al. 2006).

The results revealed quantitative differences in the protein profile between the two experimental groups (lake and sea) showing that protein electrophoresis is a sensitive tool for aquatic biomonitoring (Osman et al. 2010). It was seen that changes in biochemical parameters are evidently related to exogenous factors such as water quality and seasonal changes, but also to endogenous factors such as reproductive cycle. Fish reproduction, in particular for females, is the primary cause of fluctuations in blood parameters. Bani and Vayghan (2011) showed that, in kutum, there is a decrease in total protein levels during reproduction time. *M. cephalus* is reproductively active from October through April, and our study was performed in May, so the changes in protein levels that we found could be due to water quality.

Our results suggest that the reduction of serum protein concentration in group A could be due to protein catabolism, i.e. the process of converting blood and structural proteins to energy to meet higher energy demands on exposure to different pH values (Das et al. 2006). Moreover, these results confirm that *M. cephalus* is able to adapt to a wide range of environmental salinities and pH, while facing extra energy costs, probably related to osmoregulatory processes. Amino acids seem to play an important role in fish adjustment to different environmental salinities, as either energy sources or important osmolytes for cell volume regulation (Costas et al. 2012).

Some researchers have shown that the concentrations of total protein, albumin and globulin in plasma represent indicators of liver function (Berneta et al. 2001). The decrease of serum protein could be attributed to renal excretion or impaired protein synthesis, or due to liver hypofunction or disorder (Kori-Siakpere 1995). On the other hand, the observed decrease of serum protein could also result from breakdown of protein into amino acids first and possibly into nitrogen and other elementary molecule. Osman et al. (2010) found that, in the African catfish, the alterations in protein patterns can be attributed to SOM-induced inhibition of protein synthesis. In agreement with these authors, our results suggest that, in *M. cephalus*, mainly changes of SOM and CHL*a* acted as stressors, promoting alteration of liver function and thus protein synthesis.

The results of our research provide a further contribution to the knowledge of haematological parameters and the electrophoretic pattern of *M. cephalus*, which is a fish suitable for biotests, and emphasise the fact that changes in blood characteristics are important indices for monitoring the effects of habitat changes on fish physiology.

The data obtained with mullet can be considered as useful references for comparison of the biomarker responses of organisms living in different aquatic habitats, clearly showing that protein electrophoresis is a sensitive tool for aquatic biomonitoring. However, further studies comparing haematological parameters and electrophoretic patterns together with biochemical parameters of *M. cephalus* collected from different sites are needed.

Acknowledgments The authors thank the members of SEAC diagnostic group for their scientific, technical and logistic support. 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. The authors would like to thank the Province of Messina for allowing the sampling.

References

Andrade VM, Freitas TRO, Silva J (2004) Comet assay using mullet (*Mugil* sp.) and sea catfish (*Netuma* sp.) erythrocytes for the detection of genotoxic pollutants in aquatic environment. Mutat Res 560:57–67

Atamanalp M, Yanik T, Haliloglu HÏ, Sitki AM (2002) Alterations in the haematological parameters of rainbow trout, *Oncorhynchus mykiss*, exposed to cypermethrin. Israeli J Aquaculture 54: 99–103

Bani A, Vayghan AH (2011) Temporal variations in haematological and biochemical indices of the Caspian kutum, *Rutilus frisii kutum*. Ichthyol Res 58:126–133

Bergamasco A, Azzaro M, Pulicanò G, Cortese G, Sanfilippo M (2005) Ganzirri Lake, north-eastern Sicily. In: Giordani G, Viaroli P, Swaney DP, Murray CN, Zaldívar JM Marshall Crossland JI (eds) Nutrient fluxes in transitional zones of the Italian coast, *LOICZ* Reports and Studies n° 28 LOICZ IPO, Texel, The Netherlands, pp 103–110

Berneta D, Schmidta H, Wahlia T, Burkhardt-Holm P (2001) Effluent from a sewage treatment works causes changes in serum chemistry of brown trout (*Salmo trutta* L.). Ecotox Environ Safe 48:140–147

Borkovic SS, Pavlovic SZ, Kovacevic TB, Štajn AŠ, Petrović VM, Saičić ZS (2008) Antioxidant defence enzyme activities in hepatopancreas, gills and muscle of spiny cheek crayfish (*Orconectes limosus*) from the River Danube. Comp Biochem Physiol Part C Toxicol Pharmacol 147:122–128

Cazenave J, Bacchetta C, Parma MJ, Scarabotti PA, Wunderlin DA (2009) Multiple biomarkers responses in *Prochilodus lineatus* allowed assessing changes in the water quality of Salado River basin (Santa Fe, Argentina). Environ Pollut 157:3025–3033

Christensen GM, Faindt JT, Poeschi BA (1978) Cells, proteins and certain physical-chemical properties of brook trout (*Salvelinus fontinalis*) blood. J Fish Biol 12:51–60

Costas B, Aragão C, Soengas JL, Míguez JM, Rema P, Dias J, Afonso A, Conceição LE (2012) Effects of dietary amino acids and repeated handling on stress response and brain monoaminergic neurotransmitters in Senegalese sole (*Solea senegalensis*) juveniles. Comp Biochem Physiol A Mol Integr Physiol 161:18–26

Das PC, Ayyappan S, Jena JK (2006) Haematological changes in the three Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) exposed to acidic and alkaline water pH. Aquaculture 256:80–87

Davis AK, Maney DL, Maerz JC (2008) The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol 22:760–772

De Domenico E (1987) Caratteristiche fisiche e chimiche delle acque nello Stretto di Messina. In: Di Geronimo I, Barrier P, Mantenat C. (eds) Le Detroit de Messine, evolution tectono-sedimentaire recente (Pliocene et Quaternaire) et environment actuel. Documents et Travaux de l'Igal, Paris, 11, pp 225–235

De Pedro N, Guijarro AI, López-Patiño MA, Martínez-Álvarez R, Delgado MJ (2005) Daily and seasonal variations in haematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. Aquac Res 36:1185–1196

Depledge MH, Aagaard A, Gyorkos P (1995) Assessment of trace metal. Toxicity using molecular, physiological and behavioural biomarkers. Mar Pollut Bull 31:19–27

Deutsch HF, Goodloe MB (1945) An electrophoretic survey of various animal plasmas. J Biol Chem 161:1–20

Fernandes MN, Mazon AF (2003) Environmental pollution and fish gill morphology. In: Val AL, Kapoor BG (eds) Fish adaptation. Science, Enfield, pp 203–231

Fernandes C, Fontaìnhas-Fernandes A, Rocha E, Salgano MA (2008) Monitoring pollution in Esmoriz-Paramos lagoon, Portugal: liver histological and biochemical effects in *Liza sapiens*. Environ Monit Assess 145:315–322

Ferreira M, Moradas-Ferreira P, Reis-Henriques MA (2005) Oxidative stress biomarkers in two resident species, mullet (*Mugil cephalus*) and flounder (*Platichthys flesus*), from a polluted site in River Douro Estuary. Portugal Aquat Toxicol 71:39–48

Ferreira F, Santos MM, Reis-Henrinques MA, Vieira MN, Monteiro NM (2011) The annual cycle of spermatogenesis in *Lipophrys pholis* (Blenniidae), a recently proposed sentinel species for pollution monitoring. Ichthyol Res 58:360–365

Hongkun NA, Qingyu H, Yingying C, Huang H (2008) Differential proteins revealed with proteomics in the brain tissue of *Paralichthys* under the stress of methyl parathion. Chin J Chromatogr 26:662–666

Houston AH (1997) Review: are the classical hematological variables acceptable indicators of fish health? Trans Am Fish Soc 126: 879–894

Innamorati M, Ferrari I, Marino D, Ribera D'Alcalà M (1990) Metodi nell'ecologia del plancton marino. Nova Thalassia, Trieste

Jerônimo GA, Martins ML, Bachmann F, Greiner-Goulart JA, Schimitt-Junior AA, Ghiraldelli J (2009) Hematological parameters of *Pimelodus maculates* (Osteichthyes: Pimelodidae) from polluted and non-polluted sites in the Itajaí-Ac¸ u River, Santa Catarina State, Brazil. Acta Sci Biol Sci 31:179–183

Kekic M, dos Remedios CG (1999) Electrophoretic monitoring of pollutants: effect of cations and organic compounds on protein interactions monitored by native gel electrophoresis. Electrophoresis 20:2053–2058

Kori-Siakpere O (1995) Some alterations in haematological parameters in *Clarias isheriensis* (Sydenham) exposed to sublethal concentration of water-born lead. Bioscience Res Commun 8:93–98

Kori-Siakpere O, Ake JEG, Avworo UM (2006) Sublethal effects of some selected haematological parameters of *Heteroclarias* (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*). Int J Zool Res 2:77–83

La Rosa T, Mirto S, Favaloro E, Savona B, Sarà G, Danovaro R, Mazzola A (2002) Impact of the water column biogeochemistry of a Mediterranean mussel and fish farm. Water Res 36:713–721

Maceda-Veiga A, Monroya M, Viscorb G, De Sostoa A (2010) Changes in non-specific biomarkers in the Mediterranean barbell (*Barbus meridionalis*) exposed to sewage effluents in a Mediterranean stream (Catalonia, NE Spain). Aquat Toxicol 100: 229–237

Manganaro A, Pulicanò G, Reale A, Sanfilippo M, Sarà G (2009) Filtration pressure by bivalves affects the trophic conditions in Mediterranean shallow ecosystems. Chem Ecol 25:467–478

Mazzola A, Bergamasco A, Calvo S, Caruso G, Chemello R, Colombo F, Giaccone G, Gianguzza P, Guglielmo L, Leopardi M, Raggio S, Sarà G, Signa G, Tomasello A, Vizzini S (2010) Sicilian transitional waters: current status and future development. Chem Ecol 26:267–283

McDonough CJ, Roumillat WA, Wenner CA (2005) Sexual differentiation and gonad development in striped mullet (*Mugil cephalus* L.) from South Carolina estuaries. Fish Bull 103:601–619

Menezes S, Soraes AMVM, Guilhermino L, Peck MR (2006) Biomarker responses of the estuarine brown shrimp *Crangon crangon* L. to non-toxic stressor: temperature, salinity and handling stress effects. J Exp Mar Biol Ecol 335:114–122

Musa SO, Omoregie E (1999) Haematological changes in the mudfish, *Clarias gariepinus* (Burchell). J Aquat Sci 14:37–42

Muthukmaravel K, Kumarsamy P, Amsath A, Paulraj MG (2007) Toxic effect of cadmium on the electrophoretic protein patterns of gill and muscle of *Oreochromis mossambicus*. E-J Chem 2:284–286

Osman AGM, Mekkawy I, Verreth J, Kirschbaum F (2007a) Effects of lead nitrate on the activity of metabolic enzymes during early developmental stages of the African catfish *Clarias gariepinus* (Burchell, 1822). Fish Physiol Biochem 33:1–13

Osman AGM, Wuertez S, Mekkawy IA, Exner HI, Kierschbaum F (2007b) Lead induced malformations in embryos of the African catfish *Clarias gariepinus* (Burchell, 1822). Environ Toxicol 22:375–389

Osman AGM, Al-Awadhi RM, Harabawy ASA, Mahmoud UM (2010) Evaluation of the use of protein electrophoresis of the African catfish *Clarias gariepinus* (Burchell, 1822) for biomonitoring aquatic pollution. Environ Res J 4:235–243

Sharaf-Eldeen Kh, Abdel-Hamid NA (2002) Sublethal effects of copper sulphate, malathion and paraquat on protein pattern of *Oreochromis niloticus*. Egypt J Aquat Biol Fish 6:167–182

Strikland JDH, Parsons TR (1992) A practical handbook of sea water analysis. Bull Fish Res Board Can 167:310

Vanucci S, Bruni V, Pulicanò G (2005) Spatial and temporal distribution of virioplankton and bacterioplankton in a brackish environment (Lake of Ganzirri, Italy). Hydrobiologia 539:83–92

Wahbi OM, Shalaby SM, El-Dakar AY (2004) Effects of pulp and paper industrial effluent on some blood parameters, gonads and flesh proteins in experimentally exposed striped sea bream *Lithognathus mormyrus*. Egypt J Aquatic Res 30:25–42