Comparative study of the biochemical and haematological parameters of four wild Tyrrhenian fish species

F. Fazio[1], S. Marafioti[1], F. Arfuso[1], G. Piccione[1], C. Faggio[2]

[1]Department of Veterinary Science, University of Messina, Messina, Italy
[2]Department of Biological and Environmental Sciences, University of Messina, Messina, Italy

ABSTRACT: A characteristic feature of fish is the wide physiological range of blood parameters and also the large individual variations. The aim of this study was to compare the haematological profile, glucose and lactate levels of four teleost fish species (*Gobius niger*, *Mugil cephalus*, *Sparus aurata*, *Dicentrarchus labrax*) and to establish the similarities and differences between these species which are widely present in the Tyrrhenian Sea. To this end, glucose, lactate and complete haematological profiles were determined for 25 fish from each species. Statistical analysis confirmed statistical differences in blood parameters among the four species. Our findings show a lower level of glucose and higher levels of lactate, red blood cells and haemoglobin in *M. cephalus* with respect to the other species. White blood cell and thrombocyte counts have the same trend and result higher in *S. aurata*. The differences found in this study can be attributed to the feeding behaviour, life style and adaptation of the different fish species to the habitat in which they dwell.

Keywords: blood parameters; glucose; lactate; teleost; sea water

Studies of blood parameters have been carried out to determine the systematic relationships among certain species (Cameron 1970; Larsson et al. 1976; Atkinson and Judd 1978; Putnam and Freel, 1978; Filho et al. 1992).

It is recognised that the blood component values exhibit genetic and physiological variations. The genetic variation may be due to interspecific factors between species and intraspecific factors within species. Changes in haematological parameters depend upon the aquatic biotope, fish species, age, and sexual maturity and health status (Blaxhall, 1972; Patriche et al. 2011; Radu et al. 2009). It is well known that blood comprises 1.3–7% of the total body weight of fish and it represents one of the most active components which, accompanied by haematopoietic organs, contributes to metabolic processes by ensuring gas exchange between the organism and the environment. For this reason, blood parameters are increasingly used as indicators of the physiological condition or sub-lethal stress response in fish to endogenous or exogenous changes (Cataldi et al. 1998; Belanger et al. 2001). The evaluation depends on the availability of reference values. These should be as close as possible to normal values of various blood components considered as reliable descriptors of healthy fish under natural conditions (Cataldi et al. 1998).

It is clear that the environment in which fish live influences the metabolic content in blood (Bullis 1993). Taking into account the long evolutionary history of bony fishes and the many adaptations to different environments, it is clear that no species can be used as a representative model for all fish.

For this study we considered four teleost fish species that are typical fish of the Tyrrhenian Sea: *Gobius niger*, *Mugil cephalus*, *Sparus aurata* and *Dicentrarchus labrax*. Normal ranges for blood parameters in these species have been established in different studies carried out on wild and farmed fish subjected to different experimental conditions (Katalay and Parlak 2004; Buscaino et al. 2010; Fazio et al. 2012a,b, 2013). The particular interest in studying the blood parameters of *G. niger* lies in the fact that this species was proposed as a good model organism because it is a territorial species that lives and feeds on the ocean floor. For this reason, these fish live in very intimate contact with their environment and are very susceptible to physical and chemical changes which may be reflected in their blood components. Also, *M. cephalus* is considered as a good sentinel organism because it has shown to be sufficiently sensitive to anthropogenic compounds in laboratory tests (Andrade et al. 2004) and therefore suitable for biomonitoring studies. It possesses several characteristics required of an estuarine sentinel species, such as extreme salinity tolerance (Ferreira et al. 2005). The study of the blood parameters of *S. aurata* and *D. labrax* is of interest due to their commercial significance, economic importance and extensive consumption as a food source.

In view of this, the purpose of the present study was to compare the haematological profiles, glucose and lactate levels of the four described teleost fish species (*G. niger*, *M. cephalus*, *S. aurata*, *D. labrax*), living in the Tyrrhenian Sea. It was hoped that an elucidation of their blood parameters may provide information about interspecies differences and adaptations of each species to their environmental conditions.

MATERIAL AND METHODS

A total of 100 healthy teleost fishes of four species, 25 black goby (*Gobius niger*), 25 mullet (*Mugil cephalus*), 25 sea bream (*Sparus aurata*) and 25 sea bass (*Dicentrarchus labrax*) were collected in May 2010 from the Tyrrhenian Sea. Table 1 describes fish source, feeding behaviour and habitat.

Blood samples were obtained by puncturing the caudal vein using a 20 G×1.5 syringe and collected in microtubes (Miniplast 0.6 ml, LP Italiana Spa, Milano) containing EDTA (ratio 1.26 mg/0.6 ml) as the anticoagulant agent. The time elapsing from capture to blood withdrawal was less than 5 min. After blood sampling, the fish were individually weighed to the nearest 0.01 g (Mark 2200, BEL Engineering Srl, Monza) and their fork lengths (*L*) were recorded. For the assessment of glucose and lactate in whole blood a portable blood glucose analyser (ACCU-Chek Active, Roche Diagnostics GmbH, Mannheim, Germany) and a portable blood lactate analyser (Accusport, Boehringer Mannheim, Germany) were used.

Haematological profiles were measured within 1 h after blood samples were taken using the HeCo Vet C blood cell counter (SEAC, Florence, Italy), which has already been used to investigate haematological profiles in *G. niger, M. cephalus* and *S. aurata* (Fazio et al. 2012a,b, 2013).

All samples were analysed in triplicate by the same operator. The samples exhibited parallel displacement to the standard curve. The overall intra-assay coefficient of variation was < 5%. Evaluation of the haemogram involved the determination of the red blood cell 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). All protocols 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 glucose, lactate values and haematological parameters were tested for normality using the Kolmogorov-Smirnov test. $P < 0.05$ was considered statistically significant. One-way analysis of variance (ANOVA) was used to determine significant differences in all parameters measured among the four species. All calculations were carried out using the statistical software Prism v. 4.00 (Graphpad Software Ldt., USA, 2003).

RESULTS

In Table 2 are listed the mean values ± SD of biometric data recorded in the four species studied. The results showed significant differences in glucose and lactate levels among all the species considered. In particular, sea bass showed the high-

Table 1. Description of fish source, feeding behaviour and habitat

Species	Author	Feeding	Habitat
Gobius niger	Linneus 1758	carnivores	demersal
Mugil cephalus	Linneus 1758	herbivores/detritus	benthopelagic
Sparus aurata	Linneus 1758	carnivores	demersal
Dicentrarchus labrax	Linneus 1758	carnivores	demersal

Table 2. Mean values ± SD of biometric parameters recorded in the four teleost species

Species	Biometric parameters	
	fork length	weight
Gobius niger	18.87 ± 2.12	72.46 ± 10.38
Mugil cephalus	32.13 ± 2.63	413.00 ± 47.29
Sparus aurata	19.30 ± 1.09	185.9 ± 28.87
Dicentrarchus labrax	19.96 ± 1.50	150.9 ± 35.18

est levels of both glucose and lactate, within the range of 50.40–246.5 mg/dl and 3.38–16.42 mmol/l, respectively.

The mean haematological values obtained for the four species are presented in Table 3. Specific variations among the species were detected in all haematological values. The RBC levels were highest in *M. cephalus* followed by *D. labrax* and *S. aurata* and lowest in *G. niger.* The highest Hgb concentrations were observed in *M. cephalus* followed by *S. aurata* and by *D. labrax*; the lowest levels were recorded in *G. niger.* Hct, WBC and TC exhibited the same trend and were highest in *S. aurata* and lowest in *G. niger.* With respect to erythrocyte indices the lowest values of MCH and MCHC were found in *D. labrax,* and the lowest values of MCV were observed in *M. cephalus*.

Bray-Curtis similarities were calculated among species and the results are shown in Table 4.

DISCUSSION

The haematological and biochemical characteristics of some fish species have been investigated with the aim of establishing normal blood values and ranges with respect to sex, age, size and environmental and physiological conditions (Kori-Siakpere 1985; Sowunmi 2003; Gabriel et al. 2007). Also, comparative studies on blood parameters of fish have been carried out to determine the systematic relationship among certain species.

Carnivorous fish species show an impaired ability to clear excesses in blood glucose levels (Cowey et al. 1977) and therefore have been traditionally considered as relatively glucose-intolerant species (Wilson 1994; Moon 2001). However, plasma glucose levels fluctuate (Hemre and Hansen 1998), and glucose is also essential for brain function (Soengas 2002), suggesting the existence of a gluco-sensing system in fish. In support of this hypothesis, our results showed levels of glucose to be highest in carnivorous fish and lowest in mullet (herbivore).

Blood lactate concentrations obtained for the four species show that this parameter was higher in more active fish, such as sea bass and mullet, compared to less active species. High-speed movements require large increases in muscular activity; therefore, when this exceeds the ability of the circulatory system to transport oxygen to the active tissues, anaerobic metabolism supplements the aerobic metabolism (Heath and Pritchard 1962; Brett 1972). The capacity for anaerobic energy production has been estimated from decreases in substrate reserves and product accumulation with the conversion of glucose to lactate followed by stimulation of pyruvate metabolism and oxygen debt (Puckett and Dill 1984, 1985; Kauffman 1990; Goolish 1991).

Jawad et al. (2004) found that values of RBC, Hct and Hgb increased with increasing fish size. In our

Table 3. Mean values ± SD of haematological and biochemical parameters recorded in the four teleost species

Haematological parameters	*Gobius niger*	*Mugil cephalus*	*Sparus aurata*	*Dicentrarchus labrax*
RBC (×10⁶/μl)	1.45 ± 0.23	3.73 ± 0.40	3.06 ± 0.43	3.49 ± 0.28
Hct (%)	22.47 ± 3.54	41.0. ± 3.31	53.33 ± 4.42	49.29 ± 6.17
Hgb (g/dl)	5.67 ± 0.89	11.07 ± 1.05	9.95 ± 1.06	8.90 ± 0.76
WBC (×10³/μl)	9.41 ± 1.41	23.38 ± 4.42	47.36 ± 8.91	27.22 ± 5.65
TC (×10³/μl)	30.16 ± 7.65	47.99 ± 9.12	108.20 ± 28.81	80.72 ± 19.64
MCV (fl)	159.29 ± 26.86	110.40 ± 9.84	176.30 ± 10.89	141.00 ± 11.05
MCH (pg)	40.35 ± 7.51	29.79 ± 2.06	32.99 ± 4.75	25.50 ± 1.24
MCHC (g/dl)	25.85 ± 5.79	27.02 ± 1.99	20.56 ± 2.60	18.16 ± 1.33
Glucose (mg/dl)	168.90 ± 35.39	50.40 ± 8.40	192.8 ± 47.00	246.50 ± 30.93
Lactate (mmol/l)	3.38 ± 0.62	8.84 ± 1.99	6.36 ± 1.60	16.42 ± 1.68

Table 4. Bray-Curtis similarity of the four teleost fish

Bray-Curtis similarity	*Gobius niger*	*Mugil cephalus*	*Sparus aurata*	*Dicentrarchus labrax*
Gobius niger	1	0.7048	0.81275	0.78607
Mugil cephalus	0.7048	1	0.68315	0.69693
Sparus aurata	0.81275	0.68315	1	0.87217
Dicentrarchus labrax	0.78607	0.69693	0.87217	1

study, values of RBC and Hgb were highest in mullet, which have the highest biometric parameters. It should be noted that the differences recorded in blood parameters between fish of various sizes according to Raizada et al. (1983) are genetically determined, but Chaudhuri et al. (1986) suggest that the differences might be due to the higher metabolic rate of bigger fish compared to smaller ones. Moreover Svobodova et al. (2008) reported that active species displayed higher values of haematological parameters compared to less active forms. High RBC values were associated with fast movement, predaceous nature and high activity with streamlined bodies (Rambhaskar and Srinivasa Rao 1986). Among the four species studied, *G. niger* showed the lowest RBC, HCT, and Hgb values. The Black Goby is a relatively quiet and sedentary fish; it builds its nest under shells or stones and guards its eggs there. These behaviours force the species to stay in close contact with the ocean floor. MCV, MCH and MCHC were calculated indirectly with reference to RBC, Hct and Hgb; therefore, their changes are directly linked with these blood parameters.

WBCs are the defensive cells of the body. According to Douglass and Jane 2010, their levels have implications for immune responses and the ability of the animal to fight infection. Species with higher levels of WBC will be able to fight infection more effectively than other species. Our findings showed that WBC counts seem to have wide range of variation from 9.41 to 47.36 × 10^3/μl. Among our studied species, except for *G. niger,* there was an inverse relationship between WBC and RBC counts, which perhaps, lessen the requirement for large numbers of WBCs. The same inverse relationship between WBCs and RBCs was found by Satheeshkumar et al. 2010 on seven different teleost fish including *M. cephalus.* TC count followed the same trend of WBCs, with highest values in sea bream and lowest in black goby. There are reports suggesting that fish thrombocytes have phagocytic ability and participate in defence mechanisms (Stosik et al. 2001). Fish thrombocytes represent a link between innate and adaptive immunity (Passantino et al. 2005) and express surface and intracellular molecules that are involved in immune function (Kollner 2004). It is already recognised that fish thrombocytes are blood phagocytes that form one a protective barrier (Tavares-Dias and Moraes, 2004; Prasad and Charles 2010; Prasad and Priyanka 2011). Haematological studies contribute to an understanding of the relationship between blood characteristics and the habitat and the adaptability of the species to the environment, so there is a need for establishing normal haematological values in different species of fish. This study not only confirmed the baseline data on the blood profiles of black goby, mullet, sea bream and sea bass, but from the data we were able to establish similarities and differences between these species which dwell in the Tyrrhenian Sea.

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. Mutation Research 560, 57–67.

Atkinson E, Judd FW (1978): Comparative hematology of Lepomis microlophus and Cichlasoma cyanoguttatum. Copeia 2, 230–237.

Belanger JM, Son JH, Laugero KD, Moberg GP, Dorochov SI, Lankford SE, Cech JJ (2001): Effects of short-term management stress and ACTH injections on plasma cortisol levels in cultured white sturgeon, Acipenser transmontanus. Aquaculture 203, 165–176.

Blaxhall PC (1972): The haematological assessment of the health of freshwater fish. Journal of Fish Biology 4, 593–604.

Brett JR (1972): The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. Respiratory Physiology 14, 151–170.

Bullis RA (1993): Clinical pathology of temperate freshwater and Estuarine fish. In: Stoskopf MK (ed): Fish Medicine. Saunders Company, USA. 232–239.

Buscaino G, Filiciotto F, Buffa G, Bellante A, Di Stefano V, Assenza A, Fazio F, Caola G, Mazzola S (2010): Impact of an acoustic stimulus on the motility and blood parameters of European sea bass (Dicentrarchus labrax L.) and gilthead sea bream (Sparus aurata L.). Marine Environmental Research 69, 136–142.

Cameron JN (1970): The influence of environmental variables on the hematology of pinfish (Lagodon rhomboids) and stripped mullet (Mugil cephalus). Comparative Biochemistry and Physiology 32, 175–192.

Cataldi E, Di Marco P, Mandich A, Cataudella S (1998): Serum parameters of Adriatic Sturgeon Acipenser naccarii (Pisces: Acipenseriformes): Effects of temperature and stress. Comparative Biochemistry and Physiology 120, 273–278.

Chaudhuri SH, Pandit T, Benerjee S (1986): Size and sex related variations of some blood parameters of sarotheriodon massambica. Environment and Ecology 4, 61–63.

Cowey CB, Knox D, Walton MJ, Adron JW (1977): The regulation of gluconeogenesis by diet and insulin in rainbow trout (Salmo gairdneri). British Journal of Nutrition 38, 463–470.

Douglass JW, Jane KW (eds.) (2010): Schalm's Veterinary Hematology. John Wiley and Sons, Blackwell Publishing Ltd. 1232 pp.

Fazio F, Faggio C, Marafioti S, Torre A, Sanfilippo M, Piccione G (2012a): Comparative study of haematological profile on Gobius niger in two different habitat sites: Faro Lake and Tyrrhenian Sea. Cahiers de Biologie Marine 53, 213–219.

Fazio F, Filiciotto F, Marafioti S, Di Stefano V, Assenza A, Placenti F, Buscaino G, Piccione G, Mazzola S (2012b): Automatic analysis to assess haematological parameters in farmed gilthead sea bream (Sparus aurata Linnaeus, 1758). Marine and Freshwater Behaviour and Physiology 45, 63–65.

Fazio F, Marafioti S, Torre A, Sanfilippo M, Panzera M, Faggio C (2013): Haematological and serum protein profiles of Mugil cephalus: effect of two different habitat. Ichthyological Research 60, 36–42.

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. Aquatic Toxicology 71, 39–48.

Filho DW, Elbe GJ, Cancer G, Caprario FX, Dafne AL, Ohira M (1992): Comparative hematology in marine fish. Comparative Biochemistry and Physiology 102, 311–321.

Gabriel UU, Anyanwu PE, Akinrotimi OA (2007): Blood characteristics Associated with confinement stress in Black chin Tilapia, Sarotherodon melanotheron. Journal of Fish International 2, 186–189.

Goolish EM (1991): Aerobic and anaerobic scaling in fish. Biological Reviews of the Cambridge Philosophical Society 66, 33–56.

Heath AG, Pritchard AW (1962): Changes in the metabolic rate and blood lactic acid of blue gill sun fish, Lepomis macrochirus following severe muscular activity. Physiological Zoology 38, 767–776.

Hemre GI, Hansen T (1998): Utilisation of different dietary starch sources and tolerance to glucose loading in Atlantic salmon (Salmo salar), during parr-smolt transformation. Aquaculture 161, 145–157.

Katalay S, Parlak H (2004): The effects of pollution on haematological parameters of Black Goby (Gobius niger L., 1758) in Foca and Aliaga Bays. Journal of Fisheries and Aquatic Science 21, 113–117.

Kauffman R (1990): Respiratory cost of swimming in larval and juvenile cyprinids. Journal of Experimental Biology 150, 343–366.

Kollner B, Fischer U, Rombout JHWM, Taverne-Thiele JJ, Hansen JD (2004): Potential involvement of rainbow trout thrombocytes in immune functions: a study using a panel of monoclonal antibodies and RT-PCR. Development and Comparative Immunology 28, 1049–1062.

Kori-Siakpere P (1985): Haematological characteristics of Clarias isheriensis. Journal of Fish Biology 27, 259–263.

Jawad LA, Al-Mukhtar MA, Ahmed HK (2004): The relationship between haematocrit and some biological parameters of Indian shad, Tenualosa ilisha (family Clupeidae). Animal Biodiversity and Conservation 27, 47–52.

Larsson A, Johansson-Sjobeck ML, Fanger R (1976): Comparative study of some haematological and biochemical blood parameters in the fishes from Skagerrak. Journal of Fish Biology 9, 425–440.

Moon TW (2001): Glucose intolerance in teleost fish: fact or fiction? Comparative Biochemistry and Physiology. Part B: Biochemistry and Molecular Biology 129, 243–249.

Passantino L, Cianciotta A, Patruno R, Ribaud MR, Jirillo E, Passantino GF (2005): Do fish thrombocytes play an immunological role? Their cytoenzymatic profiles and function during an accidental piscine candidiasis in aquarium. Immunopharmacology and Immunotoxicology 27, 345–356.

Patriche Tanti, Patriche N, Bocioc E, Coada MT (2011): Serum biochemical parameters of farmed carp (Cyprinus carpio). Aquaculture, Aquarium, Conservation and Legislation – International Journal of the Bioflux Society 4, 137–140.

Prasad G, Charles S (2010): Haematology and leucocyte enzyme cytochemistry of a threatened yellow catfish Horabagrus brachysoma (Gunther 1864). Fish Physiology and Biochemistry 36, 435–443

Prasad G, Priyanka GL (2011): Effect of fruit rind extract of Garcinia gummi-gutta on haematology and plasma biochemistry of catfish Pangasianodon hypophthalmus. Asian Journal of Biochemistry 6, 240–251.

Puckett KJ, Dill LM (1984) Cost of sustained and burst swimming to juvenile Coho Salmon (Oncorhynchus kisutch). Canadian Journal of Fisheries and Aquatic Science 41, 1546–1551.

Puckett KJ, Dill LM (1985): The energetics of feeding territorially in juvenile coho salmon (Oncorhynchus kisutch). Behaviour 92, 97–111.

Putnam RW, Freel RW (1978): Hematological parameters of five species of marine fishes. Comparative Biochemistry and Physiology 61, 585–588.

Radu D, Oprea L, Bucur C, Costache M, Oprea D (2009): Characteristics of haematological parameters for carp culture and Koi (Cyprinus carpio Linneaus, 1758) reared in an intensive system. Bulletin UASVM, Journal of Animal Science and Biotechnology 66, 1–2.

Raizada MN, Jain KK, Raizada S (1983): Monthly variations in the hematocrit values (PCV) in a teleost, Cirrhinus mrigala (Ham.). Comparative Physiology 8, 196–198.

Rambhaskar B, Srinivasa Rao K (1986): Comparative haematology of the species of marine fish from Visakhapatnam coast. Journal of Fish Biology 30, 59–66.

Satheeshkumar P, Ananthan G, Senthilkumar D, Khan AB, Jeevanantham K (2012): Comparative investigation on haematological and biochemical studies on wild marine teleost fishes from Vellar estuary, southeast coast of India. Comparative Clinical Pathology 21, 275–281.

Soengas JL, Aldegunde M (2002): Energy metabolism of fish brain. Comparative Biochemistry and Physiology 131, 271–296.

Sowunmi AA (2003): Haematology of the African catfish, Clarias gariepinus (Burchell, 1812) from Eleyele Reservoir Ibadan, Southwest Nigeria. Zoologist 2, 85–91.

Stosik H, Deptula W, Travnicek M (2001): Studies on the number and ingesting ability of thrombocytes in sick carps (Cyprinus carpio). Veterinarni Medicina 46, 12–16.

Svobodova Z, Kroupova H, Modra H, Flajshans M, Randak T, Savina LV, Gela D (2008): Haematological profile of common carp spawners of various breeds. Journal of Applied Ichthyology 24, 55–59.

Tavares-Dias M, Moraes FR (eds.) (2004): Hematology of Teleost Fish. Villimpress, Ribeirao Preto, Sao Paulo.

Wilson RP (1994): Utilization of dietary carbohydrate by fish. Aquaculture 124, 67–80.

Received: 2013–01–13
Accepted: 2013–10–22

Corresponding Author:

Simona Marafioti, University of Messina, Department of Veterinary Science, 98168, Messina, Italy
Tel. +39 090 350 3584, Fax +39 090 350 3975, E-mail: smarafioti@unime.it