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

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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 ($\times 10^6/\mu l$)	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 ($\times 10^3/\mu l$)	9.41 ± 1.41	23.38 ± 4.42	47.36 ± 8.91	27.22 ± 5.65
TC ($\times 10^3/\mu l$)	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.

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