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Blood and Biometric Data on an Established *Salmo trutta macrostigma* (Dumeril, 1858) Population in an Italian Stream: Preliminary Results

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Abstract.- The aim of this study was to obtain a basic knowledge of some blood haematological and biochemical characteristics of *Salmo trutta macrostigma* (*S. t. macrostigma*) captured in Anapo river, Italy. White blood cell count (WBC), red blood cell count (RBC), haematocrit (Hct), hemoglobin concentration (Hgb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), thrombocyte count (TC), triglycerides, total cholesterol, urea, creatinine, glucose and lactate were measured in blood sample from 20 adult fish (8 males and 12 females). The fork length and total weight of the individuals varied from 19.00 to 29.40 cm and from 93.00 to 343.00 g, respectively, while condition factor varied from 0.89 to 1.55 g cm^{-1}. The obtained results, showed no significant difference of biometric data between males and females. About haematological and biochemical parameters Unpaired t-Test Student between females and males of the 20 *S. t. macrostigma* showed statistically significant differences only for TC ($P<0.001$) which value was lower in females respect to males. The present study represent a preliminary investigation related to a specific seasonal period as it is known that fish metabolism is influenced by water temperature, water oxygen concentration and intensity and length of sunshine. Thus our results are useful to obtain a basic knowledge for *S. t. macrostigma* in order to monitor the health status of this fish species that today appears to be at risk of extinction.

Keywords: Biometric data, haematological and haematochemical parameters, river, *Salmo trutta macrostigma*.

INTRODUCTION

Salmo trutta macrostigma (Dumeril, 1858), known as Mediterranean trout, is a fish species whose biology is poorly understood (Gandolfi *et al*., 1991; Querci *et al*., 2013). It is distributed in northern Africa, southern Europe, western Asia and Anatolia (Alp *et al*., 2003). Populations of this fish in Sardinia, Sicily, Corsica, Western side of South and Central Italy have been identified. Schöffmann *et al*. (2007) have confirmed the presence of the native *S. t. macrostigma* in Sicily, possibly as the result of an old colonization from the Atlantic (Snoj *et al*., 2011).

In particular, in Italy, during the last century domesticated strains of *S. (trutta) trutta* were transferred to most of the freshwater basins without considering the presence of autochthonous populations of *S. t. macrostigma*. Thus, illegal methods and heavy fishing pressure, reduced spawning success caused by pollution of streams, degradation of spawning habitats, river damming and interspecific competition with introduced rainbow trout have caused a decline in the stocks of this native trout populations.

Hematologic and biochemical variables are valuable diagnostic aids in fish biology as in other vertebrate species. In particular, haematological and biochemical parameters have been widely used for the description of health status of fish (Danabas *et al*., 2010), for predicting systematic relationship and the physiological adaptations of animals. Moreover, it is well known that these parameters are useful indicators of stress, tissue damage, nutritional status, lipid metabolism and non-specific immune status (Wagner and Congleton, 2004). In addition, many studies provided the effects of a variety of environmental and physiologic factors, including seasonality, water salinity, temperature and oxygen content, age and sex of the fish on haematological and biochemical parameters (Bielek and Strauss, 1993; Cataldi *et al*., 1998; Hrubec *et al*., 2001; Sadati *et al*., 2011). Since physiological and

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biochemical characteristics of fish blood are easily modified by environmental changes (Atamanalp *et al*., 2002), these parameters can be useful as biomarkers of different aquatic habitat of fish, too (Maceda-Veiga *et al*., 2010).

Despite the well-known importance of these parameters to monitor the health status of fish, the lack of published reliable reference intervals and the bewildering diversity of fish species (Clauss *et al*., 2008) has prevented the widespread use of these important tools.

Several researchers pointed out some biochemical parameters in the liver and muscles and on sperm quality of *S. t. macrostigma* (Alp *et al*., 2003, 2005; Aras *et al*., 2009; Akpinar *et al*., 2009; Querci *et al*., 2013), but researches on haematological and biochemical parameters were never carried out.

Considering that blood parameters of *S. t. macrostigma* are not known, the aim of this study was to investigate some haematological and biochemical parameters in fish of this species collected in Anapo river in Sicily. This information is useful to give a basic knowledge of these parameters related to biometric data, sex and environment for monitoring the health status of this species at risk of extinction.

MATERIALS AND METHODS

For this study 20 *S. t. macrostigma* (8 males and 12 females) were captured. Anapo River located in the South of Siracusa city, Sicily, (37°5'10"32 N, 15°16'25"68 E), by electric fishing using an electrofisher IG200/2 (Scubla, Italy) generator with 250 W and 200 V, in January 2012 (temperature 7.5°C, sunlight 6:40 and sunset 17:40). The species *S. t. macrostigma* was recognized based on phenotypic characteristics (Kocabaş *et al*., 2011). Immediately after capture all fish were transferred in a tank and anaesthetized prior to blood sampling using 2-phenoxyethanol (99%, Merck, Whitehouse Station, New Jersey, USA) at the concentration of 400 mg/l. 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 ethylene diaminetetra acetic acid (EDTA - 1.26 mg/0.6 ml) as an anticoagulant agent and the other one without EDTA. For the assessment of Glucose and Lactate on whole blood a portable blood glucose analyzer (ACCU-Chek Active, Roche Diagnostics GmbH, Mannheim, Germany) and a portable blood lactate analyzer (Accusport, Boehringer Mannheim, Germany) were used. At the end of blood sampling sex, fork length and weight of each fish were recorded and condition factors were calculated as follows: Condition factor = (Weight X 100/lenght^3).

Careful netting and handling was implemented to minimize stress. At the end of sampling all animals returned to the wild.

The blood samples collected in EDTA tubes were used for the determination of haematological profile using an automated haematology analyzer (HeCo Vet C, SEAC, Florence, Italy) with special lysing reagent for fish (SEAC, Code 71010460), previously used to investigate haematological profile in other fish species (Fazio *et al*., 2012a,b, 2013a). Evaluation of the haemogram involved the determination of the white blood cell count (WBC), red blood cell count (RBC), haematocrit (Hct), hemoglobin concentration (Hgb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and thrombocyte count (TC). In order to evaluate the reliability of the automatic haematological analysis, the evaluation of WBC, RBC and TC were carried out with the manual method and obtained results were compared with those obtained by automatic method. No statistical differences between haematological parameters evaluated with manual and automatic method were observed. The reliability of automatic haematological analysis has been demonstrated as in our previous studies on *S. aurata* (Fazio *et al*., 2012b) and *M. cephalus* (Faggio *et al*., 2013).

Serum samples obtained from blood samples without EDTA by centrifugation at 3000 rpm for 10 min, were used for estimation of triglycerides, total cholesterol, urea, and creatinine using an automated analyzer UV Spectrophotometer (SEAC, Slim, Florence, Italy).

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

63/2010/EU.

Data obtained for biometric data, blood and serum parameters were tested for normality using Kolmogorov-Smirnov test. $P < 0.05$ was considered statistically significant. Unpaired Student's t-test was used to determine significant differences of biometric parameters (length, weights and conditions factors), haematological and biochemical parameters between males and females. Data were analyzed at 95 % confidence level and all calculations were carried out using statistical software Prism 7.00 (Graphpad Software Ldt., USA, 2003).

Table I.- Biometric data (Mean±SD) of females and males *S. t. macrostigma* examinated.

Biometric parameters	*S. t. macrostigma* (Dumeril, 1858)	
	Male (n=8)	Female (n=12)
Fork length (cm)	23.38±2.07	25.57±2.53
Weight (g)	165.00±33.67	203.00±60.90
Condition factor (g cm^{-1})	1.28±0.14	1.20±0.18

RESULTS

The results of physical parameters measured in the studied area were: temperature 9.5 °C; dissolved oxygen 10.8 mL/L and pH 8.65. On the 20 *S. t. macrostigma* examinated in the study, 8 were males and 12 females. Fork length of *S. t. macrostigma* ranged from 19.00 to 29.40 cm with a mean fork length of 24.69 cm. Weights of all examined fish ranged 93.00 to 343.00 g, with a mean weight of 188.00 g. Condition factors calculated for each fish ranged 0.89 to 1.55 g cm^{-1} with a mean value of 1.23 g cm^{-1}. No significant differences ($P<0.05$) were found in biometric data (fork length, weights and conditions factors) recorded between females and males of the twenty evaluated trout. Mean Values ± Standard Deviations (SD) of biometric data recorded between females and males of the 20 *S. t. macrostigma* are reported in Table I. Table II shows the hematological parameters and biochemical community of *S. t. macrostigma* were reported.

The application of unpaired t-test Student on hematological parameters and biochemical, glucose, lactate values between females and males of the 20 *S. t. macrostigma* showed significant difference only for TC ($P<0001$) in particular TC value was lower in females respect to males with a difference of 9.62 x 10^3/µL (Table II).

DISCUSSION

In fish, biometric indices (fork length, weight and condition factor) as the haematological and biochemical parameters may change in populations of the same species from different regions and this may be due to environmental features such as water temperature, feeding and abound nourishment (Kara *et al*., 2011).

Biometric indices are important for stabilizing the taxonomic characters of the species (Pervin and Mortuza, 2008). Length and weight data are useful and standard result of fish sampling programs. Condition factor is strongly influenced by both biotic and abiotic environmental conditions and can be used as an index to assess the status of the aquatic ecosystem in which fish live (Anene, 2005). These data are needed to estimate growth rates, length and age structures, and other components of fish population dynamics (Kolher *et al*., 1995).

Our results showed different values of biometric indices and condition factors of *S. t. macrostigma* sampled in Sicily compared to the biometric data of the same species studied in Turkey (Yüksel and Kocaman, 1998; Alp *et al*., 2003, 2005). Length values recorded from *S. t. macrostigma* studied in our study are similar to those found by Querci *et al.* (2013) on *S. t. macrostigma* collected from Central Italy. This confirms that environment, and above all geographical variations, influence the biometric indices of fish as observed by other authors (Rahman *et al*., 2013). However, the different habitat does not appear to affect the growth rate between the sexes, in fact, statistical significant differences in fork length, weight and condition factor between female and male of *S. t. macrostigma* from Sicily were not statistically significant ($P>0.05$), as found in *S. t. macrostigma* collected from Turkey (Alp *et al*., 2003, 2005).

Table II.- Haematological parameters and biochemical components of 20 *S. t. macrostigma* caught from Anapo river, Sicily.

Parameters (n= 20)	Mean ± SD	Median	95% confidence interval Mean±1.96 σ	2.5th–7.5th percentile range
Haematological				
WBC (x 10^3/µL)	19.47±2.42	20.00	18.34±20.60	18.25±20.93
RBC (x 10^6/µL)	1.78±0.29	1.78	1.64±1.91	1.60±1.98
Hct (%)	43.00±4.45	43.00	40.92±45.08	42.00±46.00
Hgb (g/dL)	10.91±1.61	11.45	10.15±11.66	10.00±11.98
MCV (fL)	247.03±41.11	232.30	227.80±266.30	214.10±267.20
MCH (pg)	62.20±9.57	61.78	57.73±66.68	53.90±70.96
MCHC (g/dL)	25.30±2.24	25.41	24.25±26.35	23.81±26.52
TC (x 10^3/µL)	45.35±5.63	45.50	42.71±47.99	40.25±51.75
Biochemical				
Triglycerides (mg/dL)	48.93±8.34	45.95	45.02±52.83	44.50±50.60
Total Cholesterol (mg/dL)	221.45±81.11	192.50	183.50±259.40	185.60±228.00
Urea (mg/dL)	16.65±2.11	14.50	13.66±15.64	13.00±16.00
Creatinine (mg/dL)	0.24±0.03	0.24	0.22±0.25	0.22±0.26
Glucose (mg/dL)	54.20±8.84	55.50	50.25±58.15	49.50±61.50
Lactate (mmol/L)	2.94±0.81	2.95	2.56±3.31	2.50±3.40

Table III.- Haematological and biochemical parameters (Mean±SD) females and males of *S. t. macrostigma* examinated.

Parameters	Males (n=8)	Females (n=12)
WBC (x 10^3/µL)	20.20±0.74	19.00±3.02
RBC (x 10^6/µL)	1.82±0.21	1.75±0.33
Hct (%)	40.88±4.15	44.42±4.21
Hgb (g/dL)	10.40±1.58	11.24±1.61
MCV (fL)	225.80±20.76	264.20±45.79
MCH (pg)	57.22±6.76	65.53±9.94
MCHC (g/dL)	25.35±1.97	25.26±2.49
TC (x 10^3/µL)	51.13±1.46	41.50±3.60**
Triglycerides (mg/dL)	45.29±7.66	51.35±8.17
Total Cholesterol (mg/dL)	198.20±17.55	237.00±102.50
Urea (mg/dL)	15.13±2.17	14.33±2.10
Creatinine (mg/dL)	0.24±0.03	0.24±0.02
Glucose (mg/dL)	57.38±6.99	52.08±8.92
Lactate (mmol/L)	3.22±0.78	2.75±0.80

***P<0.0001

About hematological and biochemical parameters, our results showed that WBC, RBC, and Urea, Glucose, Lactate, respectively, were higher in males respect to females even if statistically significant difference was observed only in thrombocyte count. It is postulated that the reason for having higher haematological and biochemical values in male fish are primary due to its being biochemically as well as nutritionally richer than the female (Vijayakumari and Murali, 2012) in which the most of the metabolites and nutrients are continuously being exhausted in the development of the ovary.

Previous studies on fish hematology revealed that interpretation of blood parameters is quite difficult since variations in the blood are caused by internal and external factors, including sex (Rashidi *et al*., 2012). It is well known that blood sampling, laboratory techniques, seasonal variations, size, genetic properties, sex, population density, lack of food supply, environmental stress and transportation can affect hematological data (Wilhem *et al*., 1992; Örün and Erdemli, 2002; Arnold, 2005; Kori-Siakpere *et al*., 2005). For this reason, researchers must be careful when establishing reference intervals and making comparisons. Often, a direct comparison is not possible because of the different experimental conditions. Hematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fish live could exert some influence on the hematological characteristics (Gabriel *et al*., 2004; Fazio *et al.*, 2013a,b). It is known that fish are poikiloterm organism and their metabolism is

influenced by water temperature, dissolved oxygen concentration and intensity and length of sunshine. The results of the present study referred to a particular season period provide basic knowledge of some haematological and biochemical data of wild *S. t. macrostigma* with specific biometric data and physical water characteristics and give useful information about possible variations related to sex. However, these findings should be considered a preliminary data due to the small number of sampled specimens and the lack of more samplings that can show the influence of seasonality on measured parameters. Therefore, further studies should be carried out to fill the lack of knowledge about this brown trout.

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Conflict of interest

The authors disclaim any financial support or relationships that may pose conflict of interest.

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