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Open Access Journal of Veterinary Science & Research
ISSN: 2474-9222

Hematological Parameters in Trout: A Comparative Study between Rainbow Trout *Oncorhynchus Mykiss* (Walbaum, 1792) and Brown Trout *Salmo Trutta Macrostigma* (Dumeril, 1858)

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Research Article

Volume 2 Issue 2

Received Date: March 03, 2017

Published Date: April 27, 2017

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Abstract

The aim of this study was to obtain a basic knowledge of some blood haematological parameters in two different species of trout, Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and Brown trout *Salmo trutta macrostigma* (Dumeril, 1858). *O. mykiss* was caught in a farming system (commercial farm Palazzolo Acreide (Siracusa, Italy) while *S. t. macrostigma* in natural habitat (Anapo river, Siracusa, 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), were measured in blood samples from a total of 40 fish (20 for each species).

The fork length, total weight and condition factor of the individuals were measured. Unpaired Student's t-test between two species showed statistically significant differences ($P < 0.001$) for RBC, Hct, MCV, MCH, MCHC and TC. No statistically significant differences were found in WBC and Hgb.

These results are useful to obtain a basic knowledge of two different species of trout in order to monitor the health status of these species of great commercial value.

Keywords: Biometric data; Haematochemical parameters; *Oncorhynchus mykiss*; *Salmo trutta macrostigma*

Introduction

Rainbow trout (*Oncorhychus mykiss*) is a fish (family of Salmonidae) that represents a species particularly reared in aquaculture because it is a resistant fish, easy to spawn, fast growing and capable of occupying many different habitats because it can tolerate a wide range of environmental and production conditions better than other trout species [1].

Brown trout *Salmo trutta macrostigma* (Dumeril, 1858), known as Mediterranean trout, is a species of fish belonging to the genus *Salmo trutta* [2] whose biology is poorly understood [3]. This species has been identified in Italy where they are widely spread in Sardinia, Sicily, Corsica, South and Central Italy [4]. Schöffmann *et al*. [5] have confirmed that the presence of the native *S. t. macrostigma* in Sicily, possibly is the

result of an old colonization from the Atlantic [6]. *S. t. macrostigma* has been included in the EU Habitats Directive (Annex II) and in the WWF (World Wildlife Fund) red list of species at risk of extinction [7].

Haematological parameters have been widely used for the description of health status of fish [8] and are useful indicators of stress, nutritional status, pathological conditions and non-specific immune status [9]. *Oncorhynchus mykiss* and *S. t. macrostigma* are two species phylogenetically very similar, but despite the well-known importance of these parameters to monitor the health status of fish, the lack of published reliable reference intervals and the diversity of fish species [10] have prevented the widespread use of these important tools. Previous ours research showed hematological parameters in this species [11,12] in a different number of fish. Furthermore, several researchers have focused the investigation on some haematochemical parameters of *S. t. Macrostigma* [4,7,13,14] and *Oncorhynchus* [15,16] but a comparative study on haematological parameters between these two species were never carried out.

Considering the lack information about blood parameters in these species the aim of this study was to investigate some haematological parameters in Rainbow Trout *O. mykiss* (Walbaum, 1792) and Brown trout *S t. macrostigma* (Dumeril, 1858) in order to give a basic knowledge of these parameters for monitoring the health status of these species of great commercial interest.

Material and Methods

For this study a total of 40 trouts (20 *O. mykiss* and 20 *S. t. macrostigma*). Twenty *O. mykiss* were caught by an Italian farm. This farm, located in Palazzolo Acreide, Siracusa (Sicily, Italy), at an altitude of 679 m above the sea level, consists of 11 open concrete rectangular tanks. Each rectangular tank was 5 m in width, 20 m in length and 3.5 m deep. The volume of each tank was 80 m^3. Fish stocking density was 25 kg/m^3 for tank.

Twenty *S. t. macrostigma* were caught by Anapo River, located in the South of Siracusa, Sicily, that comes from the southern slope of Mount Lauro, about 900 m, and flows into the Ionian Sea by the Great Harbour of Siracusa.

In the sites of sampling some chemical and physical parameters of the water (temperature, oxygen and pH) were measured by means of a multiparametric probe YSI 85 system.

All fish immediately after capture were transferred in a tank and anaesthetized prior to blood sampling using MS222 at the concentration of 0, 7 g/L. Blood samples were collected by caudal vein using a sterile plastic syringe (2.5 ml) and transferred into tubes (Miniplast 0.5 ml, LP Italiana Spa, Milano) containing ethylenediaminetetraacetic acid (EDTA - 1.26 mg/0.5 ml) as an anticoagulant agent. At the end of blood sampling fork length, weight was recorded and condition factors of each fish were calculated as follows: $W \times 100/L^3$ where W is the weight of the fish in grams (g), L is the length of the fish in centimeters (cm). For salmonids, K values usually fall in the range 0.8 to 2.0 [17].

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 these fish species [11,12] and in other [18,8,19]. 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). 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.

Statistical Analysis

Data obtained for biometric and haematological parameters were tested for normality using Kolmogorov-Smirnov test. $P < 0.05$ was considered statistically significant. Unpaired t-Test Student was used to determine significant differences of biometric parameters (length, weights and conditions factors) and haematological parameters between the two species of fish. Data were analyzed at 95 % confidence level and all calculations were carried out Data were analyzed using statistical software prism v. 5.00 (Graphpad Software Ldt., USA, 2003).

Results

Mean Values ± Standard Deviations (SD) of biometric data recorded in 20 *O. mykiss* and 20 *S. t. macrostigma* and physical parameters measured in the studied areas are reported in Table 1. In Table 2 Mean Values ± Standard Deviation (SD) and the descriptive statistics of hematological parameters obtained in 20 *O. mykiss* and 20 *S. t. macrostigma* were reported.

Water Parameters	*Oncorhynchus mykiss*	*Salmo trutta macrostigma*
Temperature (°C)	9.86±0.23[a]	9.50 °C±0.31[a]
Dissolved Oxygen (mg/L)	8.13±0.01[a]	8.07±0.05[a]
pH	8.40±0.07[a]	8.56±0.08[a]
Biometric Parameters		
Weight (g)	211.09±28.71[a]	210.04±32.60[a]
Fork length (cm)	30.95±2.91[a]	29.85±1.14[a]
Condition factor K	0.77±0.42[a]	0.79±0.10[a]

Table 1: Water quality values (Mean±DS) and biometric data of Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and Brown trout *Salmo trutta macrostigma* (Dumeril, 1858).

Means without the same alphabetical characters within the same parameters represent statistical differences ($P < 0.001$).

The application of Unpaired t-Test Student on hematological parameters between *O. mykiss* and *S. t. macrostigma* showed significant difference ($P < 0.001$) for RBC, Hct, MCV, MCH, MCHC and TC.

Discussion and Conclusion

In this study the results showed no statistically significant differences of weight, length and condition factor of *O. mykiss* respect to *S. t. macrostigma*. This is an important result because the comparative study on haematological parameters is mainly focused on the differences between the two species. It is known that weight and length can affect the studied haematological parameters [20] which are also influenced by several environmental parameters in which the species live [19,21-23]. The chemical-physical water parameters showed no statistically significant differences ($P < 0.001$), so, the results obtained can be discussed in relation to the differences between the two species.

Our results have shown in the two species of trout that several parameters (RBC, Hct, MCV, MCH, MCHC and TC) are significantly different ($P < 0.001$). In particular *O. mykiss* RBC, Hct and MCV values were significantly lower than *S. t. macrostigma* instead MCH, MCHC and TC have shown statistically significant higher values (Table 2). No statistically significant differences were found in WBC and Hgb. It was demonstrated that the differences obtained in blood parameters between different species are genetically determined [24]. Moreover Svobodova [25] reported that active species displayed higher values of haematological parameters compared to less active forms. The high RBC, Hct and MCV values obtained in *S. t. macrostigma* respect to *O. mykiss* are in according to the results showed by Rambhaskar and Srinivasa Rao [26] and they could be associated with fast movement, predaceous nature and high activity with stream lined bodies.

Parameters	Species	Mean ± SD	95% confidence interval	2.5th-7.5th percentile range
RBC(x 10^6/µL)	*O. mykiss*	1.53±0.13[a]	1.48±1.58	1.45±1.62
	S. t. macrostigma	1.84±0.18[b]	1.77±1.91	1.70±2.00
WBC(x 10^3/µL)	*O. mykiss*	20.10±0.94[a]	19.75±20.45	19.61±20.57
	S. t. macrostigma	19.67±1.97[a]	18.93±20.40	18.00±21.25
Hgb (g/dL)	*O. mykiss*	10.12±1.99[a]	9.37±10.86	9.00±11.68
	S. t. macrostigma	10.67±1.32[a]	10.18±11.17	10.00±11.70
Hct (%)	*O. mykiss*	29.00±3.24[a]	27.79±30.21	26.01±31.05
	S. t. macrostigma	42.47±1.98[b]	41.73±43.21	41.00±44.00
MCV (fL)	*O. mykiss*	189.3±12.25[a]	184.70±193,90	184.9±196.40
	S. t. macrostigma	232.8±26.17[b]	223.00±242.50	210.5±247.10
MCH (pg/cel)	*O. mykiss*	65.62±9.184[a]	62.19±69.05	60.88±70.73
	S. t. macrostigma	58.61±10.14[b]	54.82±62.39	51.71±64.83

MCHC (%)	*O. mykiss*	34.68±4.44[a]	33.02±36.34	32.65±37.43
	S. t. macrostigma	25.16±3.14[b]	23.99±26.34	22.73±26.82
TC (x 10^3/µL)	*O. mykiss*	52.13±11.73[a]	47.75±56.51	43.75±57.25
	S. t. macrostigma	44.60±3.77[b]	43.19±46.01	42.00±46.50

Table 2: Statistical results for the evaluated haematological parameters in Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and Brown trout *Salmo trutta macrostigma* (Dumeril, 1858). Means without the same alphabetical characters within the same parameters represent statistical differences ($P < 0.001$).

*RBC (red blood cells); WBC (white blood cells), Hgb (haemoglobin concentration); Hct (haematocrit); MCV (mean corpuscular volume); MCH (mean corpuscular haemoglobin); MCHC (mean corpuscular haemoglobin concentration), TC (thrombocyte count).

The results of the present study provide basic knowledge of some haematological data of the two species of fish *O. mykiss* and *S. t. macrostigma*. However, these findings should be considered a preliminary data due to the small number of sampled specimens. It is well known that, to make a significant scientific knowledge in order to determine a reference range in a species and then carry out a comparative study is necessary to use a large number of subjects. Therefore, further studies should be carried out to fill the lack of knowledge about trout.

Conflict of Interest

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

Acknowledgments

The authors would like to thanks the farm “La Trota”, strada Maremonti S.S. 287, Palazzolo Acreide (Syracuse), Italy, for providing samples and for collaborating during the study.

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