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Blood Hemogram Profiles of Farmed Onshore and Offshore Gilthead Sea Bream (*Sparus aurata*) from Sicily, Italy

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Abstract

The aim of the present study was to assess the values of haematological parameters using an automatic method and to evaluate the blood profile in gilthead sea bream (*Sparus aurata*) reared in different aquaculture systems (offshore cage and onshore tanks). For this reason, 100 fish (5 replicates for each aquaculture system) reared in two Sicilian farms with different management: offshore cages (Farm 1) and onshore open concrete tanks (Farm 2), were used. In both aquaculture farms, physical and chemical characteristics of water were detected. After collection, blood samples were immediately analysed with both manual and automatic methods. Our results showed that sea bream with similar biometric data (in mean 292 g in weight and 27.2 cm in total length for Farm 1; 232 g in weight and 24.6 cm in total length for Farm 2), but rearing in different conditions, have different baseline haematological values and this underline that water quality could influence haematological parameters of fish. In particular, it was seen that Farm 2 showed a lower value of RBC, Hct and WBC and higher value of Hgb, TC, MCV, MCH and MCHC than Farm 1. In particular, mean values of 3.28 and 2.89 in RBC; 7.38 and 45.19 in Hct, 8.31 and 10.66 in Hgb, 62.55 and 53.80 in WBC, 67.92 and 88.66 in TC, 146.3 and 157.5 in MCV, 25.55 and 37.13 in MCH and 17.6 and 23.62 in MCHC were recorded respectively for Farm 1 and Farm 2. The variations in haematological parameters due to different location of farm allow us to claim that changes in blood characteristics are important indices in monitoring the effect of habitat changes and management on the fish physiology.

Keywords: Gilthead sea bream, blood profile, aquaculture, offshore cage, onshore tank.

Introduction

Among the several species of marine fish, one that stands out is the gilthead sea bream, *Sparus aurata*, for its commercial interest, economic importance and extensive consumption as food source. This species shows many biological aspects that are favourable for aquaculture: it is a euryhaline fish, has gregarious habits and it is tolerant to high densities, so it is one of the major Mediterranean species produced by the aquaculture industry.

Despite sea bream shows a great potential allowing its rearing in captivity, the lack of a strong mechanism of natural resistance and acclimation in the winter makes the breeding of this species not yet ideal. Farmed gilthead sea bream are affected by a pathological condition termed ''winter disease'' or ''winter syndrome'' during long-term exposure to cold (Ibarz *et al*., 2010). Knowledge of haematological and immunological responses could contribute to the evaluation of resistance against infectious diseases and thereby enable to increase survival rate and reduce the time and cost needed for growth (Morgan *et al*., 2003; Kumari *et al*., 2006; Bowden *et al*., 2007). Even if haematological data are not used routinely in health care of fish, they are gradually introduced to determining the health status of these animals (Percin and Konyalioglu, 2008).

Assessments of blood parameters in fish have thus far been performed manually, using a haemocytometer (Gbadamosi Oluyemi *et al*., 2008). Unlike mammals, fish blood cells are nucleated and this makes calibration of and reading by automated systems difficult. Tavares-Dias *et al.* (2008) used an automatic blood cell counter for the evaluation of red blood cells (RBC) in two freshwater fish species, but all other haematological parameters such as white blood cells and thrombocyte counts were assessed using blood smear techniques. Shahi and Singh (2011) used an auto analyser to measure parameters such as hematocrit value, hemoglobin content and

white and red blood cells in *Channa punctatus* but they supplied few details of their method so it is difficult to assess the comparability of the reported results.

The haematological profile represents a good indicator of physiological dysfunctions beacause of the close association of circulatory system with the external environment (Elahee and Bhagwant, 2007; Percin *et al.*, 2010). It provides information not only about the health status of fish and the physical and chemical parameters of water in which they live, but it also helps to assess the relationship between these factors and to know the susceptibility of organism to changes in environmental conditions (Percin *et al.*, 2010; Ayoola *et al*., 2011). The study of the haematological characteristics of cultured fish species is an important tool in the development of aquaculture system, particularly in regard to the use in detection of healthy from diseased or stressed animal (O'Neal and Weirich, 2001; Percin and Konyalioglu, 2008).

Aquaculture systems consist of different farming structures that are included in two larger categories: onshore and offshore farming systems. Onshore aquaculture comprises a significant proportion of global aquaculture due to the role of the Asia Pacific region in global production (FAO 2011), but an important development is currently occurring in Europe and North America, driven by the increased interest in offshore aquaculture and made possible by improvements in rearing and culture structures (Aguilar-Manjarrez *et al*., 2008). Fish welfare in offshore farms is expected to improve due to higher water quality (Pelegri *et al*., 2006) with less influence from terrestrial run-off and coastal activities (Holmer, 2010). In fact, offshore cage are exposed to stronger currents certainly reducing bottom sedimentation and accumulation of organic matter: this promotes waste dispersal and minimizes the risk of pollution and self-pollution, thus ensuring a higher dissolved oxygen than in onshore tanks. On the contrary, most water quality problems experienced in outdoor tanks were associated with low dissolved oxygen and high fish waste metabolite concentrations in the culture water (Sanni and Forsberg, 1996).

As the aquaculture industry expands and knowledge on the factors that have effect on farmed fish, such as variations of temperature, oxygen, salinity, pH receives considerable attention, a non-lethal automatic and rapid method to monitor the health status of fish in relation to environmental conditions is the request for successful farming. In view of this, our aims were: (1) to analyze the blood profile of *Sparus aurata* using an automatic method and comparing the results with those obtained by traditional manual methods (2) to assess the haematological parameters of specimens from different aquaculture system method (offshore cage and onshore tank) (3) to evaluate the impact of the different aquaculture systems on blood profile.

Materials and Methods

Experimental Procedure

The experiment was conducted on a whole of 100 adult gilthead sea bream (*Sparus aurata:* Teleostei, Sparidae) in excellent health, taken by two different fish farms: an offshore aquaculture system (Farm 1) and an onshore aquaculture system (Farm 2).

The first farm is composed by 16 "Farmocean" sea offshore cages located 3.2 km off the North-Western Sicily coast (Italy). These semi-submergible rigid cages are designed with a rigid steel framework. The net is fixed inside the main floating hexagonal frame and its shape is maintained by a sinker tube attached to the bottom. The volume of each cage is of 3000 m^3. A feed system is placed on the top of the floating frame.

The second farm is a typical land based aquaculture system located on the South-Eastern coast of Sicily (Italy) and characterized by open concrete tanks. Each rectangular tank is 5 m in width, 20 in length and 3.5 m deep.

Two groups of sea bream were used in the present study; 50 animals (Farm 1) in total were taken from 5 cage of the offshore farm (5 replicate groups of 10 animals) and 50 animals (Farm 2) (5 replicate groups of 10 animals) from 5 concrete tanks of the onshore farm. Both groups were captured in January 2011. Fish stocking density was 37.2 and 37.5 kg/m^3 for cage and tank respectively.

After capture, each replicate of Farm 1 was placed in a separate tank (500 litres) positioned on a boat and containing water taken from the cage; replicates of Farm 2, instead, were placed in separate tanks (500 litres) positioned close the onshore concrete tanks. For Farm 1, just after the landing, the tank was moved to the floor near a mobile laboratory in which the equipment required for the blood sampling and the haematological analyses was located. For Farm 2, a mobile laboratory for the haematological analyses was placed near the tank.

The fish of both groups were anaesthetized prior to blood sampling using 2-Phenoxyethanol (99%, MERCK, Whitehouse Station, NJ, USA) at the concentration of 400 ppm and successively underwent venipuncture for blood collection. In total, 14 biometric parameters of each individual fish were assessed. Table 1 shows mean values ± SD of the measured biometric parameters. Blood samples were obtained from the puncture of caudal vein using a 18 G×1 ½ syringe and collected into micro tubes (Miniplast 0.6 ml, LP Italiana Spa, Milano) containing EDTA (1.26 mg/0.6 ml) as an anticoagulant agent. All samples were immediately frozen and maintained in this condition until the analysis.

Quality of Water

In both aquaculture farms, physical and chemical characteristics of water were detected five times during the entire month of January 2011: it was done before, during and after the experimental fish collection (Table 2). Temperature, salinity, dissolved oxygen, pH were measured using a CTD multiprobe (model 556 MPS, YSI – Ohio, USA) at a depth of two meters. Moreover, other parameters were measured in water samples of 10 ml collected at a depth of two meters with a 1.7 litres PVC Niskin bottle (KC Denmark A/S, Silkeborg - Denmark). No filtration was employed, nutrient samples were stored at –20°C and nitrate, nitrite, orthosilicate and orthophosphate concentrations were determined using a Brän–Luebbe Auto Analyzer following classical methods (Grasshoff *et al*., 1999).

Automatic Haematological Analysis

All samples were analysed in duplicate by the same operator immediately after collection. The samples exhibited parallel displacement to the standard curve. The overall intra-assay coefficient of variation was < 5%. The analytical procedure was performed in order to determine the following blood parameters: 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).

Hematological parameters were assessed using the blood cell counter HeCo Vet C (SEAC, Florence, Italy) with a method already used both in *Sparus aurata* than in other fish species (Faggio *et al*., 2012; Fazio *et al*., 2012a, 2012b; Fazio *et al*., 2013).

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.

Manual Haematological Analysis

In order to validate the reliability of the automatic method, a manual haematological analysis was performed on all samples (N= 100) 1 hour after collection.

RBC counts were done manually using a Neubauer haemocytometer (Shah and Altindag, 2005). Particularly, 20 µl of each whole blood sample was diluted with 0.98 ml of Dacie's fluid (1 ml of 40% formaldehyde i.e., full strength, 3.13 g trisodium citrate, 0.1 g brilliant cresyl blue, dissolved in 100 ml

Table 1. Biometric parameters (Mean ±SD) of farmed sea bream (*Sparus aurata*) of Farm 1 (n=50) and Farm 2 (n=50)

Biometric parameters	Units	Farm 1	Farm2
		Mean±SD	Mean±SD
Weight	g	292.0±72.3	232.5±78.1
Total lenght	cm	27.2±3.6	24.6±3.4
Fork lenght	cm	25.0±2.8	22.7±2.9
Muscolar body lenght	cm	22.6±2.5	20.8±2.6
Head lenght	cm	6.0±0.6	5.6±0.6
Maximum height	cm	8.8±0.8	8.1±0.9
Minimum height	cm	2.1±0.4	1.9±0.1
Distance between floor height and floor caudal	cm	18.7±1.9	17.2±1.7
Distance between nose and floor height	cm	8.5±1.4	7.6±1.7
Condition Factor (wight/lenght ratio)	%	10.6±1.6	9.2±1.8
Visceral somatic index	%	5.4±1.0	6.1±1.0
Liver somatic index	%	1.4±0.3	1.7±0.4
Gonadosomatic index	%	0.4±0.1	0.3±0.2
Spleen somatic index	%	0.1±0.0	0.1±0.0

Table 2. Water quality values (Mean±SD) for the two farms assessed during the month of January 2011

Parameters	Farm 1	Farm 2
Temperature (°C)	15.47±0.29	16.90±0.57
Water Salinity (ppt)	36.37±0.30	38.00±0.51
Dissolved Oxygen (mg/dl)	10.61±0.58	6.00±0.29*
pH	6.72±0.30	8.20±0.23*
NO_3 (µM)	0.13±0.005	2.22±0.02*
NO_2 (µM)	0.10±0.01	0.80±0.05*
PO_4 (µM)	0.32±0.01	2.40 ±0.002*
NH_3 (µM)	0.80 ±0.03	1.28±0.02*
SiO_2 (µM)	0.60 ±0.03	1.18±0.01*

Significance: * vs Farm 1 P<0.05

of distilled water). The solution was gently mixed to disperse the cells. This provided a 1:50 dilution of the blood. The mixed solution was drawn into a disposable plastic pipette. Discard the first few drops, and touch one drop to the edge (between the cover slip and counting chamber) of a Neubauer haemocytometer. Capillary action draws the sample under the cover slip (Handy and Depledge, 1999). The haemocytometer is divided into 9 areas of 1 mm^2. The central area is further divided into 25 secondary squares (volume of 0.004 mm^3), which in turn contain a grid of 16 squares. RBC were counted on microscope in 5 of the secondary squares (model DM750, Leica Microsystems GmbH- Wetzlar, Germany) at 640 X. The calculation is as follows:

Average cells count from the squares X 50/volume of square. RBC are expressed as $10^6/mm^3$.

WBC and TC were counted by using a Neubauer hemocytometer (Shah, 2010). Blood was diluted 1:20 with Turk's diluting fluid (1 per cent glacial acetic acid solution and Gentian violet 0.3 per cent w/v dissolved in distilled water) and four large (1 sq mm) corner squares of the hemocytometer were counted on microscope (model DM750, Leica Microsystems GmbH- Wetzlar, Germany) at 640 X. The total number of WBC and TC are expressed as $10^3/mm^3$ PCV was determined by microhematocrit centrifugation. Microcapillary tubes were filled, plugged with clay, and centrifuged at 19.000g for 5 minutes. Measure the length of the columns containing packed red cells, and packed red cells plus supernatant. The calculation of hematocrit is as follows: (packed red cells/packed red cells plus supernatant)/100%

Hb concentration was measured with Hb test kit (Roach GmbH Mannheim, Germany) using the cyanmethemoglobin method. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated indirectly by the above direct parameters values using standard formulas as follows:

$MCV = Hct \times 10/RBC$, $MCH = Hb/RBC$ and $MCHC = Hb \times 100/Hct$.

Statistical Analysis

The Kolmogorov–Smirnov test was used to evaluate the normal distribution of the water chemical-physical, biometric and haematological data. All biometric and haematological parameters did not present statistical differences among the 5 replicate groups at each Farm and showed a CV less than 18%. To validate the reliability of the automatic method, a Paired t-test was applied between the haematological parameters obtained by manual and automated methods for both groups (Farm 1 and 2).

Moreover, unpaired T-tests were used to assess statistical differences of biometric values, water chemical and physical parameters, and automatic haematological responses measured between Farm 1 and 2.

P-values of <0.05 were considered statistically significant. All statistical analyses were performed using the STATISTICA 7.0 (StatSoft) software package.

Results and Discussion

As reported in Figure 1 and 2 no statistical differences were observed between haematological parameters evaluated with manual and automatic methods in both groups.

Haematological parameters found in both groups (Figure 3) are in a range just reported by Ibarz *et al*. (2010). Unpaired t-test showed statistical differences in all haematological parameters between two groups except for Hct value. Fish reared in Farm 1 showed higher levels of RBC and WBC and lower levels of Hgb, TC and eritrocytes indices respect to fish reared in Farm 2. In particular, mean values of 3.28 and 2.89 in RBC; 7.38 and 45.19 in Hct, 8.31 and 10.66 in Hgb, 62.55 and 53.80 in WBC, 67.92 and 88.66 in TC, 146.3 and 157.5 in MCV, 25.55 and 37.13 in MCH and 17.6 and 23.62 in MCHC were recorded respectively for Farm 1 and Farm 2.

No significant differences were found in all biometric parameters between two groups (Table 1). On the contrary, statistical significant differences were found in chemical-physical water parameters of two aquaculture farms. As shown in Table 2, dissolved oxygen was significantly higher in offshore farm respect to onshore farm, while all other parameters resulted higher in Farm 2. The fish haematology is often hampered by the lack of reliable reference values and of standardized collection and measuring techniques (Kori-Siakpere *et al*., 2005). Manual procedures are commonly used for determining fish haematology (Pavlidis *et al*., 2007) because all fish blood cells are nucleated. Moreover, in fish it is difficult to count the WBC because they have a similar morphology to thrombocytes and for these analytical difficulties, some authors included thrombocytes with leucocytes (Ueda *et al*., 1997). Automated haematology instruments are used for mammalian blood analysis but, until now, there has been a lack of accurate automated methods available for the analysis of fish blood. This study attempts to fill this gap and make usable an automated method for the assessment of the haematological profile of gilthead sea bream. Our haematological results are in accordance with the data of values showed by Fazio *et al.* (2012a). They assessed the haematological parameters considered in this study on specimens of *Sparus aurata* very similar in weight and size to those sampled here and using the same automatic analysis system. These considerations show the reliability of the analysis method adopted in our study in this fish species.

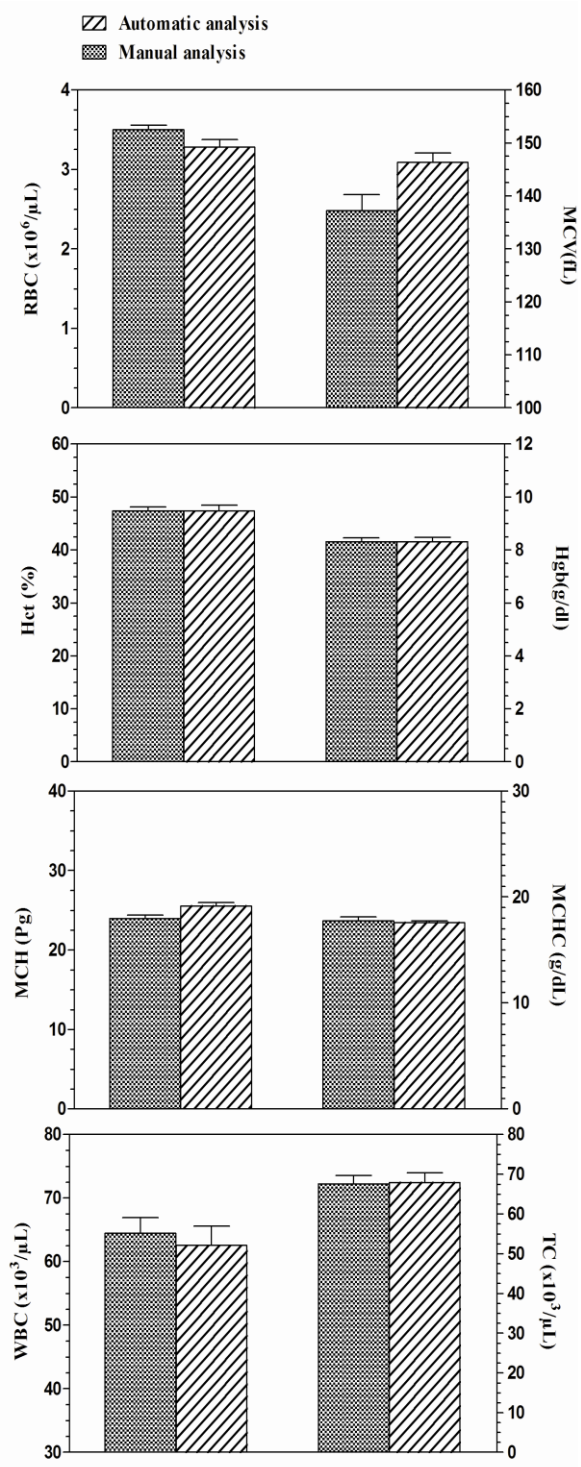

Figure 1. Mean ± SEM of haematological parameters evaluated with manual and automatic methods on gilthead sea bream (n=50) reared in sea offshore cages off the North-Western Sicily coast (Farm 1).

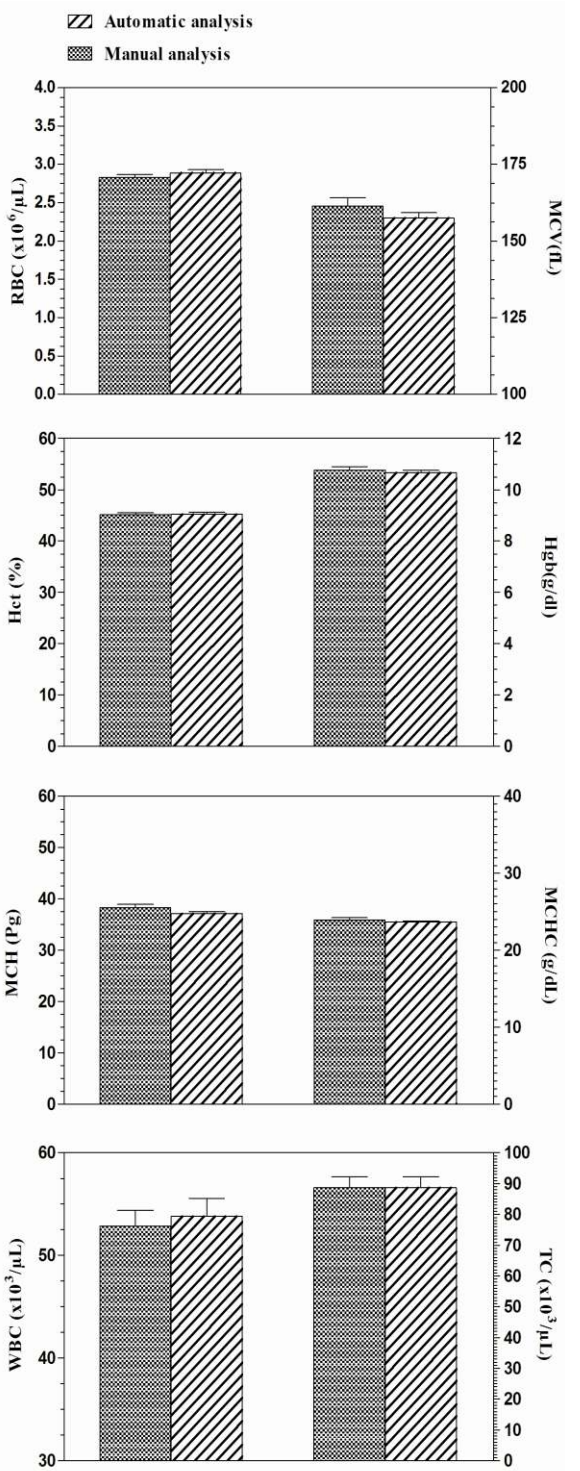

Figure 2. Mean ± SEM of haematological parameters evaluated with manual and automatic methods on gilthead sea bream (n=50) reared in onshore concrete tanks of the South-Eastern coast of Sicily (Farm 2).

In the present study, the differences among the values of haematological parameters observed in Farm 1 and Farm 2 do not allow us to relate them to the fish stocking density that showed similar values in the two farms (37.2 *vs.* 37.5 kg/m^3).

Different considerations should be made about the effects of the physic-chemical characteristics of water detected in the two aquaculture systems on the haematological parameters measured in the two fish groups. Hematological parameters are closely related

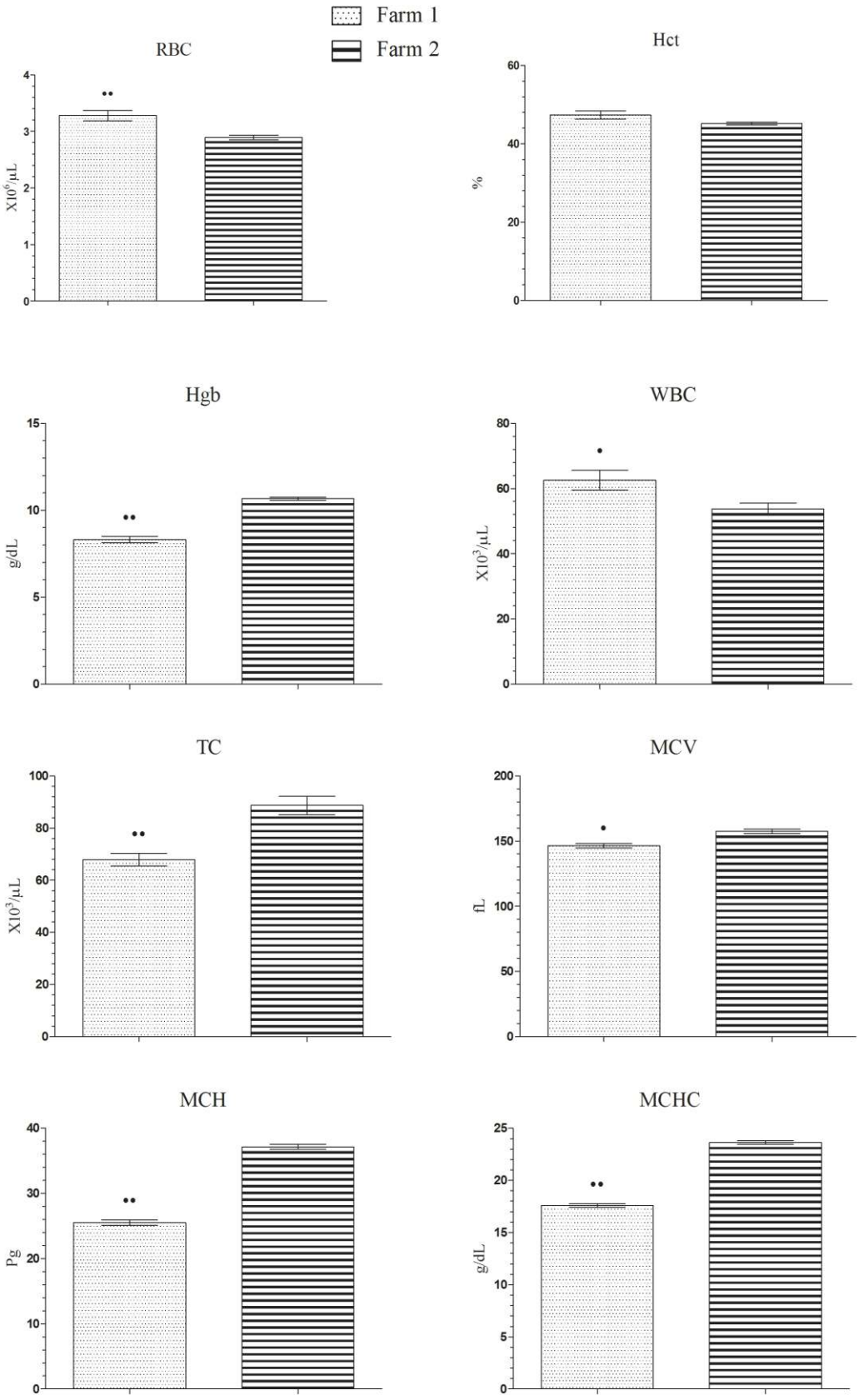

Figure 3. Mean ± SEM of haematological parameters analysed with automatic method in Farm 1 and Farm 2 with their statistically significance.

Farmed 1 vs Farmed 2; ● = P < 0.05; ●● = P < 0.01

to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on the hematological characteristics (Gabriel *et al*., 2004; Percin and Konyalioglu, 2008).

Our results showed that some parameters, such as salinity, temperature didn't show significantly differences, while other showed significant changes in offshore farm respect to onshore farm. In particular, in the latter it was observed a decrease in dissolved oxygen (DO) and increase in NH_3, NO_2, NO_3, PO_4, SiO_4 concentrations. The water quality in tanks depends on different factors such as the source, the level of recirculation, the species being reared and the wastewater treatment process within the system (Sanni and Forsberg, 1996;). Most water quality problems experienced in tanks were associated with low dissolved oxygen and high fish waste metabolite concentrations in the culture water (Sanni and Forsberg, 1996). In any aquaculture system the DO concentrations in the culture water is one of the most important parameters to be kept at safe levels, in order to provide optimal conditions for the fish (Timmons *et al*., 2002; Pillay and Kutty, 2005). The fish create and expel various nitrogenous waste products through gill diffusion, gill cation exchange, and urine and faeces excretion; in addition, some nitrogenous wastes are accumulated from the organic debris of dead and dying organisms, uneaten feed, and from nitrogen gas in the atmosphere (Timmons *et al*., 2002). Waste metabolites production includes total ammonia nitrogen (TAN), unionised ammonia (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N) and non-biodegradable organic matter. Our results showed that sea bream with similar biometric data, but rearing with different aquaculture system, have different baseline haematological values. In particular, it was seen that Farm 2 showed a lower value of RBC and Hct and higher value of MCV and Hgb than Farm 1. These findings underline that water quality influences haematological parameters and in particular in this species, the increase in MCV and Hgb could be due to compensatory mechanism to balance the low value of RBC. This is probably due to lower water quality found in onshore farm respect to offshore farm. Decreases in circulating erythrocytes in *O. niloticus* exposed to ammonia were reported by Ahamed *et al*. (1992) and Ishikawa *et al.* (2007) that found the decrease Hct too. MCHC is a measure of the concentration of hemoglobin in a given volume of packed red blood cells and it is calculated by dividing the hemoglobin by the hematocrit. So, high concentration obtained in MCHC in Group B is due to decrease in Hgb that could be due to its decrease synthesis. The lower value of WBC found in fish reared in group B farm indicate a weakened defence in the fish reared in tanks where water quality is lower than in offshore cage.

This confirms that intensive rearing have an effect on the substances involved in the natural defence mechanisms in fish (Caruso *et al*., 2005).

In conclusion, this study provides basic knowledge on the haematological values of *S. aurata* reared in onshore and offshore aquaculture systems. It evidences the reliability of an automatic method for haematological analysis in this species, highlighting its importance for use in rapid and large-scale investigations of the condition of fish.

The variations in haematological parameters due to different location of farm and different rearing management emphasize the fact that changes in blood characteristics are important indices in monitoring the effect of habitat changes and management on the fish physiology.

Therefore, establishing a baseline of information, as this work contributes to, on fish blood profile as a monitoring tool for aquaculture systems may improve the welfare and production of farmed fish. Finally, it would be useful to carry out future studies to test the use of the automatic for the blood profile assessment on more fish species from different rearing conditions.

References

Ahamed, N.A., El-Serafy, S.S., El-Shaey, A.A.M. and Abdel-Haimide, N.H. 1992. Effect of ammonia on some haematological parameters of *Orcheomis niloticus.* Proceedings of the Zoological Society A R Egypt, 23: 155-160.

Aguilar-Manjarrez, J., Kapetsky, J.M. and Soto, D. 2008. The potential of spatial planning tools to support the ecosystem approach to aquaculture. FAO Fisheries and Aquaculture Proceedings, 17. FAO, Rome, 176 pp.

Ayoola, S.O., Kuton, M.P., Idowu, A.A. and Adelekun, A.B. 2011. Acute toxicity of Nile Tilapia (*Orechromis niloticus*) juveniles exposed to aqueous and ethanolic extracts of *Ipomoea aquatica* Natural Science, 9: 91-99.

Bowden, T.J., Thompson, K.D., Morgan, A.L., Gratacap, R.M.L. and Nikoskelainen, S. 2007. Seasonal variation and the immune response: a fish perspective. Fish and Shellfish Immunology, 22: 695–706. doi: 10.1016/j.fsi.2006.08.016

Caruso, G., Genovese, L., Maricchiolo, G. and Modica, A. 2005. Haemotological, biochemical and immunological parameters as stress indicators in *Dicentrarchus labrax* and *Sparus aurata* farmed in offshore cages. Aquaculture International, 13: 67-73. doi: 10.1007/s10499-004-9031-5

Elahee, K.B. and Bhagwant, S. 2007. Hematological and gill histopathological parameters of three tropical fish species from a polluted lagoon on the west coast of Mauritius. Ecotoxicology and Environment Safety, 68: 361-371. doi: 10.1016/j.ecoenv.2006.06.003

Faggio, C., Casella, S., Arfuso, F., Marafioti, S., Piccione, G. and Fazio, F. 2012. Effect of storage time on haematological parameters in mullet, *Mugil cephalus*. Cell Biochemistry and Function, 31(5): 412-416. doi 10.1002/cbf.2915.

FAO 2011. Cultured Aquaculture Species Information Programme. www.fao.org/fishery/culturedspecies/search/en.

Fazio, F., Filiciotto, F., Marafioti, S., Di Stefano, V., Assenza, A., Placenti, F., Buscaino, G., Piccione, G. and Mazzola, S. 2012a. Automatic analysis to assess hematological parameters in farmed gilthead sea bream (*Sparus aurata* Linneaus,1785). Marine Freshwater Behaviour and Physiology, 45: 63-73. doi: 10.1080/10236244.2012.677559

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

Fazio, F., Marafioti, S., Torre, A., Sanfilippo, M., Panzera, M. and Faggio, C. 2013. Haematological and serum protein profiles of *Mugil cephalus*: effect of two different habitats. Ichthyological Research, 60: 36-42. doi: 10.1007/s10228-012-0303-1

Gabriel, U.U., Ezeri, G.N.O. and Opabunmi, O.O. 2004. Manual RBC counts were error proved and suggested that Influence of sex, source, health status and HCT and Hb may be better parameters for the assessment acclimation on the hematology of *Clarias gariepinus* of anemia in fish. African Journal of Biotechnology, 3: 463-467.

Gbadamosi Oluyemi, K., Adeparusi, E.A. and Olanrewaju, J. 2008. Basic haematogical parameters in African catfish, *Clarias gariepinus* (Burchell, 1822) FED ascorbic acid supplemented diets. Research Journal of Animal Science, 2: 17-21.

Grasshoff, K., Kremling, K. and Ehrhardt, M. 1999. Methods of seawater analysis, 3rd edition, Weinheim, Wiley-VHC. doi: 10.1002/9783527613984

Handy, R.D. and Depledge, M.H. 1999. Physiological responses: their measurement and use as environmental biomarkers in ecotoxicology. Ecotoxicology, 8: 329-349. doi: 10.1023/A:1008930404461

Holmer, M. 2010. Environmental issues of fish farming in offshore waters: perspectives, concerns and research needs. Aquaculture Environment Interactions, 1: 57–70. doi: 10.3354/aei00007

Ibarz, A., Padrós, F., Gallardo, A.M., Fernádez-Borraàs, J., Blasco, J. and Tort, L. 2010. Low-temperature challenges to gilthead sea bream culture: review of cold-induced alterations and 'Winter Syndrome'. Reviews in Fish Biology and Fisheries, 20: 539-556. doi: 10.1007/s11160-010-9159-5

Ishikawa, N.M., Ranzani-Paiva, M.J.T., Lombardi, J.V. and Ferreira, C.M. 2007. Hematological parameters in Nile Tilápia, *Oreochromis niloticus* exposed to sub-letal concentrations of mercury. Brazilian Archives of Biology and Technology, 50: 619-626. doi: 10.1590/S1516-89132007000400007

Kori-Siakpere, O., Ake, J.E.G. and Idoge, E. 2005. Haematological characteristics of the African snakehead, *Parachacnna obscura*. African Journal of Biotechnology, 4: 527-530.

Kumari, J., Sahoo, P.K., Swain, T., Sahoo, S.K., Sahu, A.K. and Mohanty, B.R. 2006. Seasonal variation in the innate immune parameters of the Asian catfish *Clarias batrachus*. Aquaculture, 252: 121–127. doi: 10.1016/j.aquaculture.2005.07.025

Morgan, A.L., Thompson, K.D., Porter, M.J.R., Burrells, C. and Bromage, N.R. 2003. Effect of seasonality on the immune response of rainbow trout. European Association of Fish Pathologists Conference, Malta.

O'Neal, C.C. and Weirich, C.R. 2001. Effects of low level salinity on production and haematological parameters of channel catfish, Ictalurus punctatus reared in multicrop ponds. In: Book of abstract. Aquaculture 2001. Int. Triennal Conf of World Aquaculture Soc Jan 21-25. Disney Colorado Springs Resort Lake Buena Vista, Florida, 484 pp.

Pavlidis, M., Futter, W.C., Katharios, P. and Divanach, P. 2007. Blood cell profile of six Mediterranean mariculture fish species. Journal of Applied Ichthyology, 23: 70–73. doi: 10.1111/j.1439-0426.2006.00771.x

Pelegri, J.L., Marrero-Diaz, A. and Ratsimandresy, A.W. 2006. Nutrient irrigation of the North Atlantic. Progress in Oceanography, 70: 366–406. doi: 10.1016/j.pocean.2006.03.018

Percin, F. and Konyalioglu, S. 2008. Serum biochemical profiles of captive and wild northern bluefin tuna, (*Thunnus thynnus* L. 1758) in the Eastern Mediterranean. Aquaculture Research, 39: 945-953. doi: 10.1111/j.1365-2109.2008.01954.x

Percin, F., Konyalioglu, S., Firat, K. and Saka, S. 2010. Serum electrolytes of wild and captive bluefin tuna (*Thunnus thynnus* L.) in Turkish Seas. Journal of Animal and Veterinary Advances, 16(9): 2207-2213.

Pillay, T.V.R. and Kutty, M.N. 2005. Aquaculture, Principles and Practices. Blackwell Publishing Ltd, Oxford, UK, 624 pp.

Sanni, S. and Forsberg, O.I. 1996. Modelling pH and carbon dioxide in single-pass seawater aquaculture systems. Aquaculture Engineering, 15: 91–110. doi: 10.1016/0144-8609(95)00003-8

Shah, S.L. 2010. Hematological changes in *Tinca tinca* after exposure to lethal and sublethal doses of Mercury, Cadmium and Lead. Iranian Journal of Fisheries Sciences, 9: 434-443.

Shah, S.L. and Altindag, A. 2005. Alteration in the immunological parameters of tench (*Tinca tinca* L.) after acute and choric exposure to lethal and sublethal treatments with mercury, cadmium and leal. Turkish Journal of Veterinary and Animal Science, 29: 1163-1168.

Shahi, J. and Singh, A. 2011. Effect of bioactive compounds extracted from euphorbious plants on hematological and biochemical parameters of *Channa punctatus*. Revista do Instituto de Medicina Tropical de Sao Paulo, 53: 259-263.

Tavares-Dias, M., Moraes, F.R. and Imoto, M.E. 2008. Haematological parameters in two neotropical freshwater teleost, *Leporinus macrocephalus* (Anostomidae) and *Prochilodus lineatus* (Prochilodontidae). Bioscience Journal, 24: 96-101.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T. and Vinci, B.J. 2002. Recirculating Aquaculture Systems. Cayuga Aqua Ventures, Ithaca.

Ueda, I.K., Egami, M.I., Sasso, W.S. and Matushima, E.R. 1997. Estudos hematologicos em *Oreochromis* (Tilapia) *niloticus* (Linnaeus, 1758) (Cichlidae, Teleostei) – part I. Brazilian Journal of Veterinary Research and Animal Science, 34: 270–275.