Assessment of haematological parameters range values using an automatic method in European sea bass (*Dicentrarchus labrax* L.)

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Abstract. This study assessed an automatic method for the definition of the range values and provided reference intervals of the haematological parameters for European sea bass (*Dicentrarchus labrax*). A manual analysis was performed on blood samples to validate the reliability of the automatic method.

Keywords: *Perciformes*, haematological parameters, European sea bass, reference values, automatic analysis, aquaculture system.

INTRODUCTION

Aquaculture plays an important role in global efforts towards eliminating hunger and malnutrition by supplying fish and other aquatic products rich in protein, essential fatty acids, vitamins and minerals. So, if the fish farmers want to improve or document fish welfare, they need methods to assess animal welfare that can be feasible on a farm. The European sea bass (*Dicentrarchus labrax*) is one of the main Mediterranean fish species produced by the aquaculture industry in Italy, with the annual production of about 9.200 t (ISMEA 2009).

Fishes are the most numerous (27,000 species) and diverse of all vertebrate groups, making generalizations regarding haematology difficult, if not impossible. One of the difficulties in assessing the state of health of natural fish population has been the paucity of reliable references of the normal condition (Kori-Siakpere et al., 2005; Satheeshkumar et al., 2011). Haematologic changes often are an early indicator of pathological changes and aid in disease diagnosis. Haematological data often are not maximally utilized in fish medicine because of the lack of reference interval (Tripathi et al., 2004). Although fish haematology continues to offer the potential of a valuable tool, progress in establishing normal range values for blood parameters has been sparse and literature in this area is scanty and often incomplete (Kori-Siakpere et al., 2005). Haematological studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. A multitude of intrinsic and extrinsic factors cause normal and abnormal variations in haematologic data (Clauss

et al., 2008) such as species and strain (Langston et al. 2002), temperature (Langston et al., 2002; Magill & Sayer, 2004), age (Svetina et al., 2002), stress (Cnaani et al., 2004; Hofer et al., 2000; Leonardi & Klempau, 2003; Morales et al., 2005), photoperiod (Leonardi & Klempau, 2003), nutritional state (Lim & Klesius, 2003; Svetina et al., 2002), the cycle of sexual maturity, health condition (Rey Vazquez & Guerrero, 2007) and water quality.

Periodic blood analyses provide an easy way of evaluating stress, metabolic disorders, reproductive dysfunctions and disease caused by environmental and husbandry conditions in cultured fish (Bayunova et al., 2002; Cech & Crocker, 2002; Furne´ et al., 2009; Hamlin et al., 2007; Lankford et al., 2003).

Recent studies on the assessment of normal blood values for some important species highlighted the importance of this knowledge as a prerequisite for evaluating health status in wild and cultured fish populations (Asadi et al., 2006 a, b, 2009; Fanouraki et al., 2007; Fazio et al., 2012b; Knowles et al., 2006; Manera & Britti, 2006; Shahsavani et al., 2008; Shi et al., 2006; Tavares-Dias & Moraes, 2007). However, the studies carried out to assess and define reference values for blood parameters in fish so far were performed manually, using a haemocytometer (Gbadamosi Oluyemi et al., 2008; Huffman et al., 1997; Kori-Siakpere et al., 2005). Only recently, Fazio et al. (2012a) measured the haematological parameters (RBC, Hb, Hct, WBC, TC, MCV, MCH and MCHC) in farmed gilthead sea bream (*Sparus aurata*) using an automatic method and Tavares-Dias et al. (2008) used an automatic blood cell counter for the evaluation of red blood cells (RBC) in two neotropical freshwater teleost species although the other haematological parameters (white blood cells and thrombocyte counts) were assessed using blood smear techniques.

In consideration of the growth of the world aquaculture industry, standardized nonlethal and automatic methods of monitoring the health status of fish will be needed. In view of this, the present study aimed: to provide an automatic method for the determination of blood parameter ranges in order to allow rapid, large-scale investigations of fish health and to define reference intervals for the future monitoring of its health and welfare under rearing conditions.

MATERIAL AND METHODS

Experimental procedure

During the month of June 2011, twenty five sub adult European sea bass (*Dicentrarchus labrax*) (Fig. 1) in excellent health, 9 months old, weighing 137.86 ± 30.33 g and 25.40 ± 1.65 mm in total length (mean ± SD), were collected from an onshore aquaculture plant of Sicily (Italy). Because haematological parameters of animals can be affected by age and size, the biometric data from fishes sampled were recorded to define their physical characteristics (Table 1).

Fishes were collected from a tank with a stocking density was 37.5 kg/m^3. Before starting the experimental procedure, the physical and chemical characteristics of the surface seawater inside the concrete tank were determined. Temperature, salinity, dissolved oxygen and pH were measured using a CTD multiprobe (model 556 MPS, YSI - Ohio, USA) at a depth of two meters. Temperature of 21.47 °C, Salinity of 38.0 ppt, Dissolved Oxygen of 7.51 mg/L and pH of 7.03 were recorded.

A mobile laboratory in which the equipment required for the blood sampling and the haematological analyses, was located close to the tank. After capture, the fish were placed in

Fig. 1 – Image of *D. labrax*, the main Mediterranean fish species produced by the aquaculture industry in Italy.

a 400l PVC tank and anaesthetized prior to blood sampling using 2-phenoxyethanol (99%, MERCK, Whitehouse Station, NJ, USA) at the concentration of 400 mg/l and successively underwent venipuncture for blood collection. Blood samples were obtained by puncturing the caudal vein using a 20G X 1 ½ syringe and collected in microtubes (Miniplast 0.6 ml, LP Italiana Spa, Milan) containing EDTA (ratio 1.26 mg/0.6 mL) as the anticoagulant agent. All samples were analyzed immediately (T0) by an automatic method (Fazio et al., 2012a).

Automatic haematological analysis

All samples were analyzed by the same operator immediately after collection. The automatic analytical measurements, performed using the blood cell automatic cell counter HeCo Vet C (SEAC, Florence, Italy) already used to assess haematological profile in other fish (Fazio et al. 2012a, b), were made in order to determine Red Blood Cell Count (RBC), Haemoglobin concentration (Hb), Haematocrit value (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cell Count (WBC) and Thrombocyte Count (TC).

Tab. 1 – Biometric parameters (Mean ±SD) of farmed European sea bass (*D. labrax*) analyzed (n = 25).

Biometric parameters	**Units**	**Mean ± SD**
Weight	g	137.86 ± 30.33
Total lenght	cm	25.40 ± 1.65
Fork lenght	cm	23.64 ± 1.74
Visceral weight	g	14.08 ± 3.60
Condition Factor (weight/lenght ratio)	%	1.03 ± 0.09
Visceral somatic index (visceral weight/weight ratio)	%	10.25 ± 1.75

To validate the reliability of the automatic method, blood samples were analyzed also with a manual analysis protocol.

Instead, using the manual analysis protocol, the following techniques were adopted: Red Blood Cells (RBC) were counted using a Neubauer haemocytometer (Shah & Altindag, 2004). Blood samples was diluited 1:200 with Hayem's fluid (Mishra et al., 1977) and erytrocytes were counted in the loaded haemocytometer chamber and the total numbers were reported as 10^6 mm^{-3} (Wintrobe, 1967); White blood cells (WBC) and thrombocyte count (TC) were obtained using an improved Neubaur haemocytometer (Shah & Altindag, 2004). Blood was diluited 1:20 with Turk's diluting fluid and placed in a haemocytometer. The total number of WBC and thrombocyte was calculated in mm^3 X 10^3 (Wintrobe, 1967); Hb concentration was measured with Hb test kit (Roach GmbH Mannheim, Germany) using the cyanmethemoglobin method (Larsen & Snieszko, 1961); MCV, MCH and MCHC were calculated indirectly by the above direct parameters values using standard formulas as follows:

MCHC ¼ Hb X 100 = Hct
MCH ¼ Hb = RBC
MCHC ¼ Hb X 100 = Hct

RESULTS

No statistical differences were observed between haematological data resulting from both manual and automatic methods (*t*-test, $p > 0.05$) (Fig. 2).

General statistics of the haematological parameters obtained by the automatic method and their reference ranges estimates are given in Table 2. They were calculated on the whole data set.

Reference intervals were calculated using parametric (mean values ± 1.96r) or non-parametric (2.5^{th} and 7.5^{th} percentiles) statistics, respectively, for variables normally and non normally distributed according to Fanouraki et al. (2007).

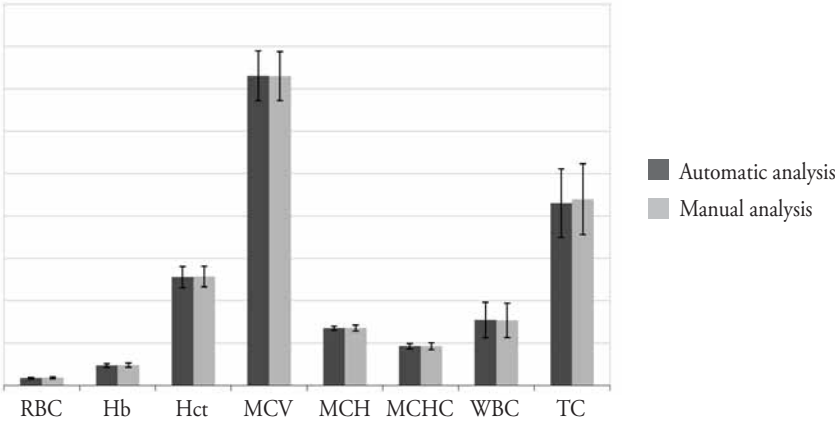

Fig. 2 – Mean ± SEM of Haematological parameters evaluated with manual and automatic methods in European sea bass (n = 50). No statistical differences were observed between manual and automatic methods (*t*-test, $p > 0.05$).

Tab. 2 – Statistical results and reference range estimate for haematological parameters evaluated with an automatic analysis in farmed sea bass (*D. labrax*) (n = 25).

Parameters	Units	Med	SD	SEM	Mean	2.5th-7.5th percentiles range	Minimum	Maximum
RBC	$x^{106/\mu L}$	3.570	0.3365	0.06731	3.510	3.315-3.730	2.680	4.040
Hb	g/dL	9.700	0.8271	0.1654	9.480	9.250-10.10	7.300	10.40
Hct	%	51.70	4.997	0.9994	51.18	47.20-55.25	40.40	58.20
MCV	fL	144.0	11.71	2.343	146.2	139.5-154.0	116.0	168.0
MCH	pg	27.20	0.9781	0.1956	27.06	26.20-27.55	25.20	29.40
MCHC	g/dL	18.40	1.250	0.2500	18.60	17.90-19.40	16.40	22.30
WBC	$x^{103/\mu L}$	31.70	8.351	1.670	30.90	23.45-36.75	13.50	45.70
TC	$x^{103/\mu L}$	87.00	16.17	3.234	86.08	71.00-96.50	60.00	113.0

DISCUSSION

Although sea bass is one the most cultured fish in Europe, there are surprisingly few reports of normal blood values. In addition, published values are severely limited by the few analytes measured.

Evaluation of haematologic and blood chemistry analytes will enhance the culture of fish by facilitating early detection of infectious disease and identification of sublethal conditions affecting production performance. Moreover, the importance of establishing the reference values is also determined by the need to understand the physiological changes of blood parameters that occur in response to environmental and biological factors. Despite recent developments in fish medicine, the interpretation of fish haematology is often hampered by the lack of meaningful reference values and of standardized collection and measuring techniques (Kori-Siakpere et al., 2005), and by the bewildering diversity of fish species (Clauss et al., 2008).

Automated haematology instruments are used for mammalian blood analysis but there has been a lack of accurate automated methods available for the analysis of fish blood. Manual procedures are commonly used for determining fish haematology (Esteban et al., 2000; Handy & Depledge, 1999; Pavlidis et al., 2007) because all fish blood cells are nucleated. Moreover, as described by Fazio et al. (2012), in fish it is difficult to count the WBC because they have a similar morphology to thrombocytes.

In the present study, the analyses confirmed the reliability of the automatic method in the species considered, showing no statistical differences between the haematological data obtained with the manual and the automatic analysis. On this regard, Fazio et al. (2012a) analyzed the same haematological parameters in sea bream (*Sparus aurata*) using the automatic method but until now, no analysis was performed on European sea bass (*Dicentrarchus labrax*). For the first time the haematological profile of European sea bass, including WBC and TC was assessed, and the reference intervals were provided using an automatic analysis. Because haematological parameters of animals can be affected by age, size, sex, quality of water, and culture conditions, the results of this study could provide haematological reference values

(Table 2) for farmed *D. labrax* with specific biometric (Table 1) and physico-chemical water characteristics.

In conclusion, the present study provides knowledge on 8 haematological parameters of *D. labrax* and highlights the importance of an automatic method for the definition of haematological reference ranges so as to make rapid, large-scale investigations of fish possible. Further studies should be conducted to assess the haematological parameters of other wild and farmed fish species, which are useful for evaluating both the health and welfare status of these animals.

Acknowdgements. The authors acknowledge the members of SEAC diagnostic group for their scientific, technical and logistic support.

RIASSUNTO

Individuazione di un range di riferimento di alcuni parametri ematologici nella Spigola (*Dicentrarchus labrax* L.) mediante metodo automatico

Il presente studio ha avuto l'obiettivo di individuare un range di riferimento per alcuni parametri ematologici nella spigola d'allevamento (*Dicentrarchus labrax* L.) mediante il ricorso ad un sistema automatico d'analisi utilizzato già in altre specie animali e recentemente testato in orate d'allevamento. Allo scopo sono stati saggiati 25 esemplari sub-adulti di spigola, in eccellente stato di salute, prelevati da una vasca per l'ingrasso di un impianto d'acquacoltura onshore della Sicilia orientale. Dopo essere stati prelevati dalla vasca, gli animali sono stati posti in un tank della capacità di 400 litri posizionato in prossimità della vasca. Successivamente, tutti gli esemplari sono stati sottoposti ad anestesia ed al prelievo ematico dal peduncolo caudale. I campioni sono stati quindi analizzati immediatamente dopo la raccolta in un laboratorio mobile allestito all'interno dell'impianto d'acquacoltura. Le analisi sono state effettuate utilizzando il contaglobuli automatico HeCo Vet C (SEAC, Firenze) al fine di determinare i seguenti parametri ematologici: Conta Cellulare dei Globuli Rossi (RBC), Concentrazione Emoglobinica (Hb), Valore Ematocrito (Hct), Volume Corpuscolare Medio (MCV), Emoglobina Corpuscolare Media (MCH), Concentrazione Emoglobinica Corpuscolare Media (MCHC), Conta Cellulare dei Globuli Bianchi (WBC) e Conta Trombocitaria (TC). Inoltre, nel sito di cattura sono stati effettuati campionamenti di acqua al fine di valutarne i principali parametri chimico-fisici (temperatura, ossigeno disciolto, salinità e pH) mediante l'utilizzo di una sonda multiparametrica (YSI 85 System). Per valutare l'attendibilità del metodo d'analisi automatico, preliminarmente al presente studio è stata effettuata un indagine sul profilo ematologico di 25 spigole provenienti dal medesimo allevamento, adottando un'analisi manuale. Dallo studio preliminare non è emersa alcuna differenza significativa tra i dati ematologici ottenuti con il metodo manuale e quello automatico. I risultati ottenuti forniscono per la prima volta i range di riferimento per alcuni parametri ematologici nelle spigole d'allevamento contraddistinte da specifiche indicazioni biometriche e mantenute in acque caratterizzate dal punto di vista chimico-fisico. Tali informazioni contribuiscono al miglioramento dei sistemi di valutazione delle condizioni di salute e benessere di questa specie sia in contesti d'allevamento che eventualmente in ambiente naturale.

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