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Sex related differences in haematological parameters in cultured striped bass (Walbaum, 1752).

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Introduction

In recent years, health monitoring of fish has gained enormous importance (Iaria et al., 2019), and the study of blood parameters of cultured fish species is a very practical and useful tool to enable aquaculture systems advance (Fazio, 2019). The growing attention to fish haematology is also due to the relationship between haematological parameters and aquatic environmental pollution (Fazio et al., 2019a; 2019c; Sayed et al., 2019). Various exogenous factors such environmental as conditions, water pollution, and stress (Nikoo et al., 2010; Gabriel et al., 2011), and endogenous factors such as age, sex and fish species (Svetina et al., 2002;

Nikoo 2012) influence et al., haematological parameters fish in values. Numerous studies have been conducted on the influence of these factors, and in particular of sex on blood parameters in different species of fish (Pourali Motlagh et al., 2012). Hybrid striped bass is a fish which is widely reared in Sicily (Santulli and Modica, 2009) where it represents one of the most emerging important aquaculture freshwater species raised for commercial use, as it is easy to breed and has a rapid growth rate.

Our study was undertaken to compare haematological parameters in male and female of hybrid striped bass to complete a haematological study of this species, which has recently been conducted with innovative diagnostic systems (Fazio *et al.*, 2019b).

Materials and methods

For haematology, the fish samples came from a Sicilian farm located in Acate (Ragusa, Sicily, Italy). In the present investigation a total of 80 adult hybrid striped bass of 2 years of age from each sex (\bigcirc 535.20±29.56g weight and 33.76±0.66 cm total length; \bigcirc 547.50±24.78g weight and 34.16±1.26 cm total length) was utilized during the natural reproductive period (Curry Woods and Sullivan, 1993).

The fish were bred in a recirculating aquaculture system (RAS) containing 22 tanks with 8m in diameter and 60m³ in volume; and a stocking density of 20 Kg⁻ 3, under conditions of seasonal temperature and constant water parameters. The water parameters in recirculating aquaculture system were measured using handheld а multiparameter instrument (model YSI 556 MPS-Ohio, USA) (Table 1). Water filtration was carried out with drum and biological filters, and an anti-bacterial UV system (200-280 nm). All fish were fed twice a day with a commercial feed and were starved a day before sampling; the fish were considered healthy based on an external examination for any sign of abnormalities or infestation. Prior to blood sampling, fish were anesthetized using tricaine methanesulfonate, TMS, MS-222 (Tricaine-S, Aqualife TMS) as reported by Iaria et al. (2019). Immediately after anaesthetization, the fish were individually weighted using a balance (Kern 440-49 N, Germany) and total length was recorded using an ictiometer (Scubla SNC, 600 mm, Italy). Blood samples were collected from individual fish in the same day and were obtained from a puncture of the caudal vein using a sterile plastic syringe (2.5 mL). All haematological parameters were determined immediately after blood collection using the automatic blood cell counter HeCo Vet C (SEAC, Florence, Italy) and were analysed in triplicates by the same operator. Protocols of animal husbandry 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 2010/63/EU for animal experiments.

Statistical analysis

The data are expressed as mean \pm standard deviation (SD) in both male and female fish and are averages of the three analyses carried out by the same operator. Unpaired *t*- test statistical method was applied to compare each parameter in the two sexes. Statistical analysis of the data was performed using the statistical software program Prism v. 7.00 (Graphpad Software Ltd, USA, 2003) (Table 1).

Table 1: Mean waterstandarddevi	ation in the
recirculating aq (RAS).	uaculture system
Water Parameters	RAS
Temperature (°C)	23.50 ± 0.10
Dissolved Oxygen (mg/L)	5.20 ± 0.01
Oxygen saturation	60%
NH ₃	0.01 mg/L
NO_2	0.2 mg/L
NO ₃	100 mg/L

Results and discussion

The results of haematological analyses are shown in Table 2. Mean values of

WBC, MCH and TC differed significantly between male and female hybrid striped bass. The values of WBC were higher in females than male fish. On the contrary, MCH and TC results showed significantly higher values in males than female hybrid striped bass. The studied biometric indices and the haematological parameters did not show significant difference comparing the two sexes (Table 2).

 Table 2: Mean values of haematological parameters and biometric indices obtained from male and female striped bass *M. saxatilis* (n = 80 per sex, ± standard deviation).

<u>temale striped bass M. saxatuls ($n = 80$ per sex, \pm standard deviation).</u>			
Haematological parameters	Mean±SD		
	Male (🖒)	Female (♀)	
WBC (x 10 ³ /µL)	$16.09 \pm 7.86^{*}$	$20.25 \pm 4.62^{*}$	
RBC ($x10^{6}/\mu L$)	2.55 ± 0.91	2.63 ± 0.49	
Hb (g/dL)	6.20 ± 1.99	6.13 ± 1.24	
Hct (%)	30.95 ± 12.04	30.89 ± 7.25	
MCV (fL)	120.70 ± 10.64	118.10 ± 11.50	
MCH (pg)	$24.95 \pm 2.92^{*}$	$23.46 \pm 2.56^{*}$	
MCHC (%)	$20.85{\pm}2.92$	19.90 ± 1.64	
TC (x 10 ³ /µL)	53.89±15.06**	$34.03 \pm 9.89^{**}$	
Biometric indices			
Weight (g)	535.20 ± 29.56	547.50 ± 24.78	
Total length (cm)	33.76 ± 0.66	34.16 ± 1.26	

(Hb) haemoglobin concentration, (Hct) haematocrit, (MCH) mean corpuscular haemoglobin, (MCHC) mean corpuscular haemoglobin concentration, (MCV) mean corpuscular volume, (RBC) red blood cells, (TC) thrombocytes, (WBC) white blood cells, * significant difference between samples (p<0.05), ** highly significant difference between samples (p<0.0001), (SD) standard deviation.

A recent study showed that blood cells of female and male fish were different (Ahmed *et al.*, 2020). Males showed higher hematological values compared to females. This difference in hematology regarding the sex may be due to different oxygen demand in males and females, which might presumably be related to difference in the reproductive activity of each fish sex (Yousefzadeh and Khara, 2015). It is also attributed to high metabolic rate and potential activeness in males with respect to females (Ahmed *et al.*, 2020). Sex is one of the main factors that influence fish haematology. In the present research, we studied this influence by comparing haematological values between males and females of hybrid striped bass. Regarding biometric indices, our results showed no significant differences in respect to sex. While significant differences were observed in some haematological parameters (WBC. MCH and TC). In our study WBC values were significantly higher in females compared to males; this data can indicate egg carriage stage or adverse conditions such as infection (Smith, 1986). A similar finding was reported by Acharya and Mohanty (2014) in a previous research conducted on another species of fish. Although in our study RBC values in females were higher than males, but the difference was not significant. Opposite results were previously found by Karimi et al. (2013) who conducted a study on the haematological profile of vellowfin seabream Acanthopagrus latus (Houttuyn, 1782) in which a significant difference in RBC between the two sexes was shown, with higher values in males compared to females. Contrary to what has been observed for WBC, MCH showed significantly higher values in a male compared to female hybrid striped bass. This result can be related to a greater probability of occurrence of macrocytic anaemia in males and is in accordance to what was found in another haematological study conducted on common carp Cyprinus carpio Linnaeus, 1758 (Baghizadeh and Khara, 2015). Regarding TC in hybrid striped bass, our findings showed significantly higher values in males compared to females. This data is in disagreement with what was found by

Pourali Motlagh *et al.* (2012) in *Betta splendens*. Hb, Hct, MCV and MCHC values were higher in males compared to females of hybrid striped bass, but they were not significantly different. This study revealed that the sex factor exercises a certain degree of influence on some haematological parameters of hybrid striped bass. Our results provide a contribution to the knowledge on characteristics of the haematological profile of striped bass and influence of sex on the biology of this species.

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