### **Short Communication**

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

### Fazio F.<sup>1\*</sup>; Saoca C.<sup>1</sup>; Perillo L.<sup>1</sup>; Bakhshalizadeh S.<sup>2</sup>; Natale S.<sup>3</sup>; Piccione G.<sup>1</sup>; Spanò N.<sup>4</sup>

Received: November 2020 Accepted: October 2021

- 1-Department of Veterinary Sciences, Polo Universitario Annunziata, University of Messina. 98168, Messina, Italy.
- 2-Department of Marine Science, Caspian Sea Basin Research Centre, University of Guilan, Rasht, Iran.
- 3-Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, F. Stagno d'Alcontres 31. University of Messina, 98166. Messina, Italy.
- 4-Department of Biomedical, Dental Sciences and Morphological and Functional Images. University of Messina, Italy.

Keywords: Farmed fish, Gender, Haematology, Morone chrysops X Morone saxatilis

#### 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 relationship between haematological parameters and aquatic environmental pollution (Fazio et al., 2019a; 2019c; Sayed et al., 2019). Various exogenous factors such environmental 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 important emerging 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

<sup>\*</sup>Corresponding author's Email: ffazio@unime.it

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 ( $3535.20\pm29.56$ g weight and  $33.76\pm0.66$  cm total length;  $9547.50\pm24.78$ g weight and  $34.16\pm1.26$  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 60m<sup>3</sup> in volume; and a stocking density of 20 Kg<sup>-</sup> <sup>3</sup>, under conditions of seasonal temperature and constant water parameters. The water parameters in recirculating aquaculture system were measured using a handheld 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 approved in accordance with 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 water quality values  $\pm$  standard deviation in the recirculating aquaculture system (RAS).

Water Parameters	RAS
Temperature (°C)	$23.50 \pm 0.10$
Dissolved Oxygen (mg/L)	$5.20 \pm 0.01$
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,  $\pm$  standard deviation).

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

(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.

#### Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

#### References

#### Acharya, G. and Mohanty, P.K., 2014.

Comparative haematological and serum biochemical analysis of catfishes *Clarias batrachus* (Linnaeus, 1758) and *Heteropneustes fossilis* (Bloch, 1794) with respect to sex. *Journal of Entomology and Zoology Studies*, 2(6), 191–197.

Ahmed, I., Reshi, Q.M. amd Fazio, F., 2020. The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. *Aquaculture International*, 28, 869–899. DOI:10.1007/s10499-019-00501-3.

# Baghizadeh, E. and Khara, H., 2015. Variability in hematology and plasma indices of common carp *Cyprinus*

- *carpio*, associated with age, sex and hormonal treatment. *Iranian Journal of Fisheries Sciences*, 14(1), 99–111.
- Curry Woods, L. and Sullivan, C.V. 1993. Reproduction of striped bass, *Morone saxatilis* (Walbaum), broodstock: monitoring maturation and hormonal induction of spawning. *Aquaculture Research*, 24(2), 211–222. DOI:10.1111/j.1365-2109.1993.tb00543.x.
- **Fazio, F., 2019.** Fish hematology analysis as an important tool of aquaculture: a review. *Aquaculture*, 500, 237–242. DOI:10.1016/j.aquaculutre.2018.10. 030.
- Fazio, F, Saoca, C., Ferrantelli, V., Cammilleri, G., Capillo, G. and Piccione, G., 2019a. Relationship between arsenic accumulation in tissues and hematological parameters in mullet caught in Faro Lake: a preliminary study. *Environmental Science and Pollution Research*, 26, 8821–8827. DOI:10.1007/s11356-019-04343-7.
- Fazio, F., Saoca, C., Costa, G., Zumbo, A., Piccione, G. and Parrino, V., 2019b. Flow cytometry and automatic blood cell analysis in striped Morone bass saxatilis 1792), (Walbaum, A new hematological approach. Aquaculture, 513, 734398. DOI:10.1016/j.aquaculture.2019.734 398.
- Fazio, F., Saoca, C., Sanfilippo, M.,

- Capillo, G., Spanò, N. and Piccione, G., 2019c. Response of vanadium bioaccumulation in tissues of *Mugil cephalus* (Linnaeus 1758). *Science of the Total Environment*, 689, 774–780.
- DOI:10.1016/j.scitotenv.2019.06.47
- Gabriel, U.U., Akinrotimi, O.A. and Eseimokumo, F., 2011. Haematological responses of wild Nile tilapia *Oreochromis niloticus* after acclimation to captivity. *Jordan Journal of Biological Sciences*, 4(4), 225–230.
- Iaria, C., Saoca, C., Guerrera, M.C.,
  Ciulli, S., Brundo, M.V., Piccione,
  G. and Lanteri, G., 2019.
  Occurrence of diseases in fish used for experimental research.
  Laboratory Animals, 53(6), 619–629.
  DOI:10.1177/0023677219830441.
- Karimi, Sh., Kochinian, P. and Salati, A.P., 2013. The effect of sexuality on some haematological parameters of the yellowfin seabream, Acanthopagrus latus in Persian Gulf. Iranian Journal of Veterinary Research, 14(1), 65–68. DOI:10.22099/ijvr.2013.1392.
- Nikoo, M., Falahatkar, B., Alekhorshid, M., Nematdost Haghi, B., Asadollahpour, A., Zarei Dangsareki, M. and Faghani Langroudi, H., 2010. Physiological stress responses in kutum Rutilus frisii kutum subjected to captivity. International Aquatic Research, 2(1),

55-60.

- Nikoo, M, Falahatkar, B. and Rahmani, H., 2012. Blood parameters of southern Caspian kutum, *Rutilus frisii. Journal of Applied Ichthyology*, 28(2), 293–295. DOI:10.1111/j.1439-0426.2010.01588.x.
- Pourali Motlagh, S., Mohammadi Zarejabad, A., Ghorbani Nasrabadi, R., Ahmadifar, E. and Molaee, M., 2012. Haematology, morphology and blood characteristics of male and female Siamese fighting fish (Betta Comparative splendens). Clinical Pathology, 21(1), 15–21. DOI:10.1007/s00580-010-1058-6.
- Santulli, A. and Modica, A., 2009.

  Aquaculture in Sicily: the state of the art. *Italian Journal of Animal Science*, 8(2), 829-838.

  DOI:10.4081/ijas.2009.s2.829
- Sayed, A.H., Kitamura, D., Oda, S., Kashiwada, S. and Mitani, H., 2019. Cytotoxic and genotoxic

- effects of arsenic on erythrocytes of *Oryzias latipes*: Bioremediation using *Spirulina platensis*. *Journal of Trace Elements in Medicine and Biology*, 55, 82–88. DOI:10.1016/j.jtemb.2019.06.007.
- Smith, L.S., 1986. Vvedenie v fiziologiyu ryb (Introduction to Fish Physiology). Agropromizdat Publishers, Moscow State University, Moscow, Russia, in Russian.
- Svetina, A., Matašin, Ž., Tofant, A., Vucemilo, M. and Fijan, N., 2002. Haematology and some blood chemical parameters of young carp till the age of three years. *Acta Veterinaria Hungarica*, 50(4), 459–467. DOI:10.1556/avet.50.2002.4.8.
- Yousefzadeh, F. and Khara, H., 2015.
  Changes in blood chemistry and hematological indices of *Capoeta capoeta gracilis* in relation to age, sex, and geographic location.

  Comparative Clinical Pathology, 24(4), 791–795.
  DOI:10.1007/s00580-014-1983-x.