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# Multidisciplinary hematology as prognostic device in environmental and xenobiotic stress-induced response in fish.

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*Graphical Abstract

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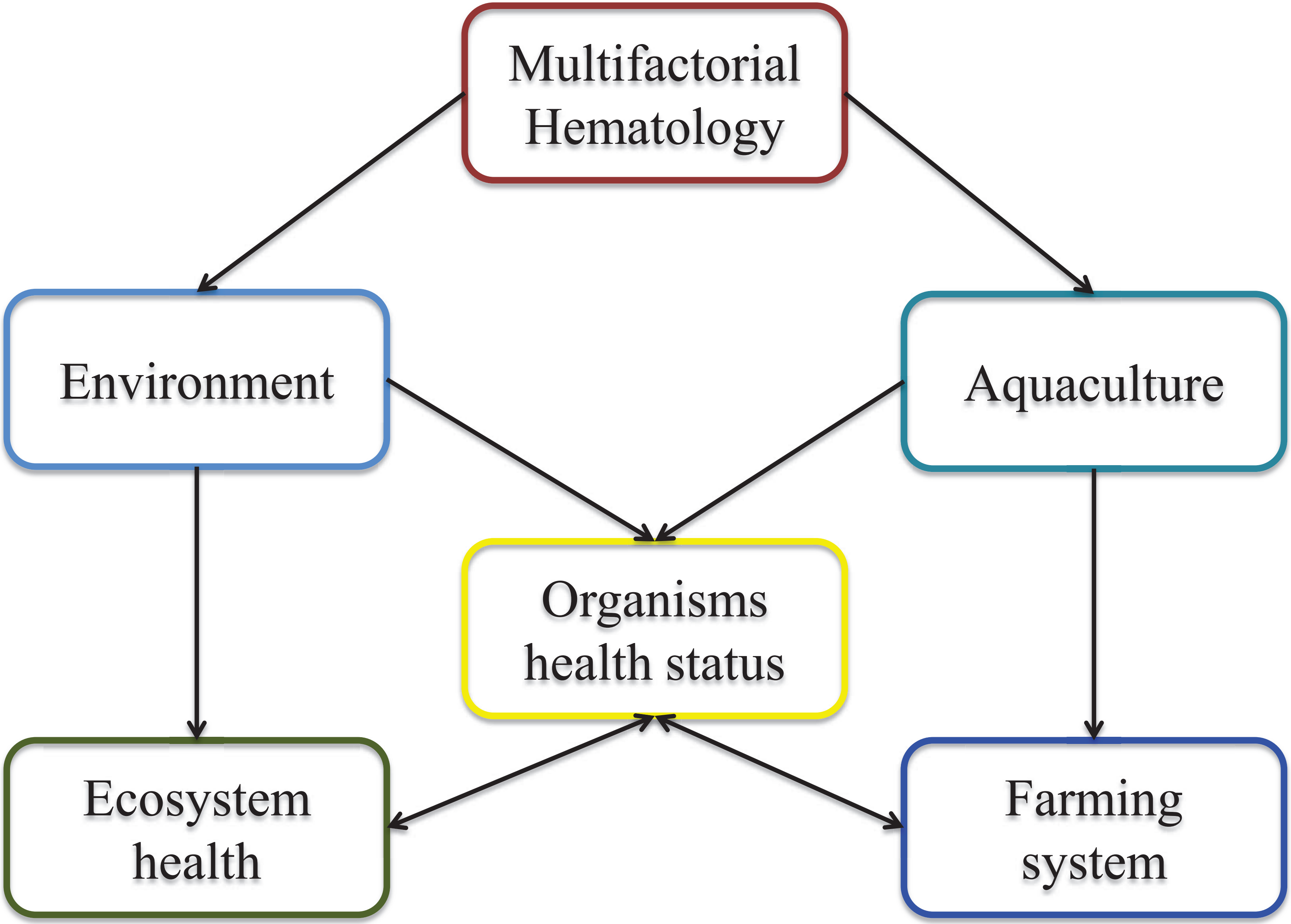

Multifactorial Hematology

Environment

Aquaculture

Organisms health status

Ecosystem health

Farming system

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## Highlights

- Hematic analysis is a good reference for environmental and aquaculture stress
- Mitochondrial bioenergetics can be a good indicator of fish health state
- Mitochondria dysfunction may be associated with the development of anemia

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# Multidisciplinary hematology as prognostic device in environmental and xenobiotic stress-induced response in fish.

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## Abstract

The use of blood components and their functions as prognostic and diagnostic tools, both in environmental studies and in aquaculture, are so important in order to understand which are the normal and pathological conditions that fish could present to a certain factors. This can allow fish health specialists to intervene before large losses occur. However, there are several factors to consider when performing a blood test, because a major limitation for field researchers is that the "rules" for animal or human hematology do not always apply to wildlife. Hence, the main of this review is to show some environmental and xenobiotic factors capable to modify the haematic cells so that it is possible to visualize the strengths and limitations of a haematological analysis underscore the problems associated with efforts to assess fish health on the basis of hematological data. Finally, we point out the importance of the use of bioenergy tools as part of haematological evaluations associated to environment or aquaculture stress.

**Keywords:** teleost; haematology analysis; environment; xenobiotic; aquaculture; bioenergetics.

## 1. Introduction

Evaluation of fish blood through hematological indices has been done for more than 70 years as a health status assessment tool and the alterations that may occur (Katz 1951; Hesser 1960; Blaxhall and Diasley, 1973). Notwithstanding, for a correct interpretation of hematological analysis is necessary to contemplate a sum of variants such as reproductive cycle, age, sex, stress, nutritional status, and water quality as well as the habitat of species, since being poikilothermic animals are under the influence of environmental changes (Bastardo and Diaz-Barberan 2005; Gabriel et al., 2004, 2007). In aquaculture as a diagnostic tool, in addition to considering the above-mentioned factors, it is necessary to consider also the sampling technique, transportation, type of culture system, acclimation procedure, and water quality (Ezeri et al., 2004; Gabriel et al., 2004, 2007, 2011; Rey Vázquez and Guerrero, 2007; Correa-Negrete et al., 2009; Faggio et al., 2014a,b,c). On the other hand, factors such as blood collection, handling and storage time of blood samples can strongly influence the results obtained from a hematological analysis, recommending carrying out the hematological evaluations immediately after blood collection because long-term storage can modifies the results of the analyses, probably due to storage-related degenerative changes that may occur (Faggio et al. 2013; Fazio et al., 2014).

A hematological study may include quantitative determinations of hematocrit (Hct), hemoglobin concentration (3Hb), Red Blood Cell (RBC) and White Blood Cell (WBC) account, platelet count (PLT), and sedimentation rate, among others. Differential blood cells (DBC) account, including RBC and WBC concentration, is

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one of the best hematological indicators of fish health because it can indicate the presence of an infectious disease (Blaxhall and Daisley, 1973) and provide data for studies of defense mechanisms disease and pathogenesis (Fijan 2002a,b). This approach has been employed in monitoring the response of fish under conditions of reproduction, nutrition, and density (Palíková et al. 1999; Pavlidis et al. 2007; Zexia et al. 2007; Burgos-Aceves et al., 2010, 2012; El-Naggar et al., 2017), or after drug administration, parasite infestations, and environmental stress (Ranzani-Paiva et al. 2008; Dias et al. 2011; Seriani et al., 2015a,b; Ventura et al., 2015; Corrêa et al. 2017; Grzelak et al. 2017; Valero et al., 2018), and thus their health status under such adverse conditions (Faggio et al., 2013). Therefore, the application of hematological indices is inexpensive and rapid to perform, allowing anticipating the clinical manifestations of diseases by monitoring the physiological, nutritional and health status of fish (Burgos-Aceves et al. 2010). Then, to have a basic knowledge of hematology represents a valuable guide to assess the condition of aquatic organisms (Rey Vázquez and Guerrero, 2007), once reference values are established under standardized conditions (Faggio et al., 2013).

## 2. Hematological indices as biomarker of environmental variations and stress

Hematological parameters are closely related to the susceptibility of animals to environment changes (Gabriel et al., 2004). Then, use of hematic tools to study fish blood composition in environmental and toxicological stress studies, as a possible indicator of physiological and pathological changes, is more recurrent (Zutshi et al., 2010; Rodrigues et al., 2018). Due to this high blood sensitivity, several blood studies have been carried out in order to understand the possible influence of seasonal changes that may have on these parameters (Folmar, 1993; Faggio et al., 2014a). A study conducted by Faggio et al. (2014a) showed that gilthead seabream *Sparus aurata* and sea bream *Dicentrarchus labrax* presented similar monthly variations trends in RBC and WBC account, Htc, and Hb, being mainly photoperiod and temperature-dependent. Both species showed an increase in values of RBC and Htc during cold-water season, which may be associated with water dissolved oxygen concentration disposal (Pascoli et al., 2011). Significant monthly fluctuations of Hb, Hct, RBC, WBC, MVC, MCH and MCHC were also reported in the Nile tilapia *Oreochromis niloticus* in an lake that experiences two seasonal period: the rainy and dry seasons (Kefas et al., 2015). The highest level of Hb, RBC, and WBC were associated to dry season, which mighty be as a result of low volume of water during this period. Whereas, MVC, MCH, and MCHC fluctuated in both rainy and dry seasons probably as consequence of agriculture activity in the area (Kefas et al., 2015). While a study done by Örün et al. (2003) in three cyprinid fish species (*Alburnoides bipunctatus F.*, *Chalcalburnus mossulensis*, *Cyprinion macrostomus*) indicates that, in addition to temperature and photoperiod, factors such as gender and water quality can influence the levels of blood parameters RBC, WBC, Hct, and Hb, adding to species factor. The three species presented the hematological indices significantly higher in the warm months that those measured in cold seasons. While the hematic values of *A. bipunctatus F.* were higher compared to those of *C. mossulensis*, *C. macrostomus*, that were similar among them. Finally, the values of RBC, Hct, ctHb, were higher in males, while a higher WBC index was reported in females, expressly during the reproductive stage. This same behavior, higher RBC values in male and higher WBC values in female, was observed in adult individuals of marine species *Mycteroperca rocasea* during the reproductive season (Burgos-Aceves et al., 2010). This increment in WBC quantity or Leukophilia in females coincided with high plasma concentrations of oestradiol (E2) and testosterone (T), while the RBC increment or erythropoietic activity in males can be associated with an increased levels of 11-ketotestosterone (11-kt) in plasma, which suggests a coordination of endocrine-immune activity (Pottinger and Pickering, 1987). In addition, it has been postulated that leukocytes can infiltrate the gonad tissue from the peripheral blood to aid with immune surveillance and phagocyte activity and, may also aid in gonad reabsorption during post-spawning. Moreover, both mature male and female presented lower levels of RBC and Hct compared to the levels reported in immature individuals, demonstrating that age is another factor that can modulate the blood parameters (Burgos-Aceves et al., 2012). While, Fazio et al. (2015) report that both haematological parameters RBC and WBC were higher in male than female of *Salmo trutta macrostigma* postulating that the reason for having higher haematological and even biochemical values in males is due to the high energy cost that females present in ovary development (Vijayakumari and Murali, 2012). Otherwise for Nile Green Tilapia *Tilapia zilli*, the nest mates presented lower levels in RBC, WBC, and Hct but without becoming significant compared with nest females (El-Naggar et al., 2017). In a comparative study, the seawater flathead grey mullet *Mugil cephalus* and the freshwater goldfish *Carassius auratus* presented significant hematological variations. Higher values of RBC and Hct, associated with reduced mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) was reported in the grey mullet in respect to goldfish. Whereas, values of WBC and Thrombocytes (TC) count were lower in the grey mullet with respect to goldfish (Fazio et al., 2012). According to previous works, high RBC values are usually associated with species of fast movement and high activity (Fazio et al., 2013a). Moreover, a high value of Hct and concomitant reduction in their volume is due to an adaptive process to salinity of seawater habitat. While, the lower levels of WBC in the seawater species could be associated to feeding habits (Satheeshkumar et al., 2012a,b; Romano et al. 2017). Thereupon, divergent environmental conditions and feeding habits may influence on fish blood parameters (Fazio et al., 2012). This means that physicochemical differences in each environment may influence the haematological parameters, which makes them suitable for monitoring the effects of habitat changes on fish biology and fish culture practices (Fazio et al., 2012).

## 3. Hematological indices as biomarker of contaminated environments

Alterations in blood parameters associated to environmental pollutants have been received growing attention in assessing the health of fish (Zutshi et al., 2010; Corredor- Santamaría et al., 2016). The variation of haematological features could serve as a biomarker of sub-lethal environmental stress (Bridges et al., 1976), since on the one hand it reflects the relative health of the aquatic ecosystem (Cazenave et al., 2005), and on the other at it can help to infer with the toxicity mode of potentially dangerous chemicals (Zutshi et al., 2010). In a report carried out by Corredor- Santamaría et al. (2016) it is emphasized that during the rain season, when industrial and domestic wastewater discharges increase, the two native species *Astyanax gr. bimaculatus* and *Aequidens metae* of a Colombian river presented alteration in the haematical parameters Hb, Hct, and RBC with a rise in WBC mainly thrombocytes and neutrophils. According to Cazenave et al. (2005), individual of neotropical freshwater fish *Corydoras paleatus* from polluted environments presented significantly higher values of RBC, Htc, ctHb, MCV, MCH and MCHC compared with individual of same species present in pristine places. Additionally, these hematic parameters did not change according to maturation stages, sex or seasons. Instead, they established that Hb could be a key parameter to point out differences between populations exposed to different environmental conditions, because an increment in Hb concentration could be an especially reliable first indicator of an adaptive improvement in blood oxygen transporting capacity (Saint-Paul, 1984). In other study, the freshwater fish *Labeo rohita* showed hematological disruptions, erythrocyte destruction (hemolysis), and leukocytosis (leukopenia) due to a synergetic effect of various pollutants present in its habitat, affecting the immune system and making the fish vulnerable to diseases (Zutshi et al., 2010). Alteration on immune system was also observed in the native Nile tilapia from an area influenced by the discharge of runoff from agricultural and urban activities. A WBC analysis denoted a high percentage of eosinophils and monocytes and fewer thrombocytes, factors that indicate poor environmental quality (Corrêa et al., 2017). The high presence of eosinophil and monocyte cells can be associates with an inflammatory response (Clauss et al., 2008; Balla et al. 2010) due to either parasite infestation or chemical compounds present in effluents (Corrêa et al., 2017). While, a reduction in thrombocytes or thrombocytopenia may be associated with internal hemorrhagic foci, which can be detrimental to the fish because these cells may be linked to inflammatory and phagocytic responses (Burrows et al., 2001; Mazon et al., 2002; Clauss et al., 2008). The read cells erythrocytes also seem to play a roll in inflammation, where deformation of these cells caused by pathogen infection or xenobiotic exposure (Pagano and Faggio, 2015; Santoso et al., 2015; Faggio et al., 2018; Farag and Alagawany, 2018; Guzzetti et al., 2018; Savorelli et al., 2017; Sehonova et al., 2018; Strungaru et al., 2019 ) seem to alter inflammation process (Straat et al., 2012). Even more, erythrocytes can play a complementary role in immune responses in both fish and other vertebrates, since it has been found that RBC expresses immunity genes and responses (Shen et al., 2018). Therefore, RBC can be used as a good indicator to evaluate the cytotoxicity of xenobiotics by membrane alteration and deformation (Pagano and Faggio, 2015; Farag and Alagawany, 2018) and could be associated to an important parameter in the study of any inflammatory response (Silva-Herdade et al., 2016).

Pollution by heavy metal in aquatic environments has been an increasing ecological and global public health concern because of the risk of toxicity and bioaccumulation in the food chain (Adeyemo et al. 2010; Aliko et al., 2015; Tchounwou et al., 2012; Fazio et al., 2014b; Pagano et al., 2017). In a study conducted by Gaber et al. (2013), individuals of African catfish *Clarias gariepinus* presented higher values of RBC, Hb, Htc and WBC in water with elevated concentration of copper (Cu), iron (Fe), lead (Pb), cadmium (Cd), manganese (Mn) and zinc (Zn) due the great discharge of wastewater by agricultural, industrial and domestic activity compared with individual of same species from a water with less sewage discharge activity. Fish of common carp exposed to Pb, Cu, Cd and Zn also presented a rise in Htc without significant changes in RBC, and an initial increase in WBC but subsequently dropped remaining low (Witeska, 2005; Vajargah et al., 2018). The increment in Htc could be translated as alarm reaction and a subsequent dewdrop as an adaptation to stress (Vosyliene, 1996). Whereas the permanence of low levels of WBC may be due to the presence of cortisol secreted that shortens the life of lymphocytes, promoting the apoptosis and reducing their proliferation (Wyets et al. 1998; Verburg van Kemenade et al., 1999; Espelid et al. 1996), The aluminum (Al) is one of the most abundant metal on earth releasing to the environment both natural or anthropogenic with no established biological functions (Sjögren et al., 2007). Due the acidification of surface waters, the aluminum becomes available to organisms that

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make it toxic to fish (Driscoll et al., 1980). In adult tropical freshwater fish *Tilapia zillii* the hematological parameters RBC, Hct, ctHb, MCHC, MCH and MCV increased significantly after aluminum exposure. These parameters increased progressively according to an increase in concentration of aluminum and time exposure, which can be a defensive mechanism against aluminum toxicity through stimulation of erythropoiesis (Alwan et al., 2009). The essential trace metal Mn is widely used in industry and its waste is dumped into water bodies becoming an indiscernible toxic metal in aquatic environment altering the physiological homeostasis of organisms. In the gold fish after an acute exposure of Mn, alterations in the blood cells were observed. A WBC differential account reveled a significant decrement of leucocytes thus compromising the immune system, while erythron profile revealed a significant increasing of cellular and nuclear alteration of red blood cells leading to eryptosis, compromising the blood oxygen carrying capacity and therefore the fish health status (Valbona et al., 2018). Mercury (Hg) is a mayor and common aquatic pollutant and can be converted into more toxic form by microbes (Schropre, 2001). It has been reported that Hg can penetrate the membrane of erythrocytes damaging the cells and causing hemorrhages as observed in the tench fish *Tinca tinca*, concomitant with elevated values of Hct, ctHb, and RBC in acute lethal or chronic sub-lethal exposure. These increments could be due a splenic contraction (a common stress response), and subsequently releases of blood cells reserve or by simultaneous erythropoiesis in response to a transport demand for $O_2$-$CO_2$ (Shah and Altindag, 2004). Notwithstanding, at lower acute sub-lethal exposure does not appear have toxic effect both tench fish (Shah and Altindag, 2004), and Nile tilapia (Ishikawa et al., 2007). The metals Cd and Pb are other two metals that both can have effects on haematological variables, reducing concentration of RBC, WBC and TC in the blood of Striped Mullet *M. cephalus* (Fazio et al., 2014b). The reduction in concentration of RBC may be associated with internal bleeding from damage to the kidney caused by Cd and Pb, in addition to an impaired osmoregulation triggered by Cd, which may cause a haemodilution (Kori-Siakpere et al. 2006; Fazio et al., 2014b). While, reduction in WBC in blood of Striped Mullet seems to be associated to a bioaccumulation of Cd and Pb in kidney and liver (Kori-Siakpere et al. 2006), weakening the immune system and, making the fish susceptible to diseases (Shah and Altindag, 2005). Similar accumulative effect of Cb in fish tissues was observed in Nile tilapia. Also, haematological parameters RBC, Hb, and Hct were reduced in fish exposed to Cd (Al-Asgah et al., 2015). According to Khadre (1988) reduction in these parameters might be due to destruction of mature RBCs and a reduction in haemosynthesis or an acute haemolytic crisis resulting in a severe anemia. Arsenic is cataloged as one of the most alarming chemicals given its high toxicity mainly in its salt form (ATSDR, 2007). Fish of Indian catfish *C. batrachus* exposed to arsenic salt presented a progressive decrease in Hb, RBC, and packed cell volume (PVC) inducing an anemia in fish (Kumar and Banerjee, 2016). Several factors may be associated with the progress of anemia, either by reduction in the read cell rate production or an increasing loss of these cells (Shah and Altindag, 2004). An accelerated destruction of hemoglobin or reduction in its rate synthesis (Reddy and Bashamohideen, 1989), a depression/exhaustion of hemopoitic potential of the fish (Sawhney and Johal, 2000), or may be a suppression of hemopoiticactivity of the kidney in addition to the increased removal of dysfunctional RBCs what decrease the PVC value following arsenic exposure (Kumar and Banerjee, 2016). According to Gill and Epple (1993) the reasons for anemia might be impaired erythropoisis caused by the direct effect of metal on kidney or spleen, accelerated erythroclasia due to altered membrane permeability and/or increased mechanical fragility, and defective iron metabolism or impaired intestinal uptake of iron due to mucosal lesions.

The use of pesticides is a worldwide practice used for control and eradication of pest in intensive agricultural production and fish farms (Oruç, 2010; Saravanan et al., 2011). The phenoxy acid herbicide (MCPA) is widely used in agriculture, forestry and horticulture (Kudsk and Streibig, 2003) and has been reported present in aquatic environments, however, little is known about its effects on fish. Lutnicka et al. (2018) evaluated the effects of this herbicide on common carp *Cyprinus carpio* juveniles presented that a chronic exposure of MCAP induces only minor and transient alterations in red blood parameters but not in leukocytes. A differential WBC count showed a significant and persistent depletion of mature neutrophils, and monocytes, indicating a possible inflammatory process and immunosuppression caused by this herbicide (Lutnicka et al., 2018). Another synthetic pesticide extensively used for controlling pests in agriculture is the Quinalphos 25EC (QP), a highly toxic organophosphate classified as a yellow label pesticide, which has become a matter of concern (Das and Mukherjee, 2000). A chronic exposure to this pesticide caused a reduction on blood parameters RBC, Htc, MCV, MCH, and MCHC as the concentration of this toxicant increased in silver barb, *Barbonymus gonionotus* (Mostakim et al., 2015). This pesticide like others has the faculty to induce histological alterations in liver and kidney but the extent of damage varies depending upon the dose of toxicants, duration of exposure, toxicity of chemical, and susceptibility of the fish (Magar and Shaikh, 2013; Shanta et al., 2013; Mostakim et al., 2015). In a previous study with common carp, Qureshi et al. (2016) pointed out that a sub-acute exposure with the pesticide fipronil and the insecticide buprofezin can induce biochemical, hematological, histopathological and genotoxic damage. At hematic level, both pesticide and insecticide (in combination or along) caused significant reduction in RBC, TC, Htc and Hb but an increment in WBC. Saravanan et al. (2011) also reported a similar effect of lindane (gamma-hexachlorocyclohexane) on haematological parameters in the same species as well as Ramesh et al. (2015) for exposure to Furadan; a carbamate pesticide is widely used in paddy fields. The levels of Hb, Hct, RBC, MCV, MCH and MCHC were decreased, whereas WBC increased in the treated fish. Such decrement in haematological parameters indicated an anemia probably due to hemosynthesis, and osmoregulatory dysfunction, erythrocyte destruction along with the damage in the gill tissues causing a reduction in oxygen carrying capacity of blood and inefficient exchange of gases (Jenkins et al., 2003; Seth and Saxena, 2003; El-Murr et al., 2015). The phenolic compound Bisphenol A (BPA), classified as potent endocrine disruptors, has detected in water environments by sewage discharges (Kamaraj et al., 2013), and has been shown to have also toxic effects in fish physiology (Liu et al., 2011; Faheem and Lone, 2013; Gentilcoreet al., 2013; Liu et al., 2014). Recently, in a work done by Krishnapriya et al. (2017), the BPA caused a significant drop in the hematological parameters Hb, Hct, MCV, and MCH with a significant increment in WBC value. The RCB value, on the contrary, presented an initial increase with a subsequent decrement. The observed reduction in hematological values may be due to a reduction in the rate of formation of erythrocytes, destruction of them and/or an anemic condition of the fish due BPA toxicity (Jenkins et al., 2003; Seth and Saxena, 2003; El-Murr et al., 2015). The increase in WBC count indicates a stimulation of the immune system against the toxicity of BPA as was also observed in yellow perch *Perca flavescens* (Rogers and Mirza, 2013). Cuesta et al. (2008) also reported a non-negative effect on the gilthead seabream head-kidney leucocytes viability with an up-regulation of some immune-

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related genes after exposure to the organochlorines 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) and lindane, exhibiting mostly a genetic effect. By contrast, it seems that in early life-history stages the Dichlorodiphenyltrichloroethane (DDT) metabolite o,p-DDE can compromise the viability of lymphocytes triggering long-term humoral immunosuppression in the Chinook salmon *Oncoryhnchus tshawytscha* (Milston et al., 2003). Same reduction in lymphocyte-granulocyte viability associated to an increasing apoptotic cells was observed in both spleen and head-kidney of Chinook salmon (Misumi et al., 2005). Notwithstanding, any information exists regarding the direct effect of DDT and/or its metabolites on fish hematology.

Despite the clear evidence of the effects of pollutants on fish hematology, it is limited to data focusing on the effects of the residual pesticides/metals on the blood system of fish, especially on the correlations between different parameters and influencing the extent of environmental factors such as pollutant concentrations or/and exposure time in each parameter (Li et al., 2011)

## 4. Hematological indices as biomarkers in fish farm aquaculture

The study of haematological characteristics in cultured fish species is an important tool in the development of aquaculture system (O'Neal and Weirich, 2001; Percin and Konyalioglu, 2008; Mauri et al., 2011). It is necessary to know the basic environmental factors that influence on fish health (Bosisio et al., 2017), which they are traditionally been based on the conditions found in its natural habitat (Deacon and Hecht, 1996). Temperature has consistently been identified as the primary abiotic factor controlling key physiological, biochemical and life-history processes in fish (Beitinger and Fitzpatrick 1979). Therefore, knowledge of fish-thermal interaction is of fundamental importance to aquaculturist (Deacon and Hecht, 1996). How fish respond to changes in temperature can be evaluated through haematological parameters since cell number, maturation grade, etc. are factors that restrict the hematic cell responses (Houston et al., 1996). The rainbow trout *Oncorhynchus mykiss* during warm periods haematological parameters Hb, Hct, and RBC are slightly lower than in cold season, which can be linked to an elevate $O_2$-carrying capacity, and $0_2$ demand. These responses appear to be anti-adaptive or, at best, neutral (Tun and Houston 1986; Houston et al., 1996). Meanwhile, leucocyte population increased significantly during the warm period, and decreased for cold period as was also observed in common carp (Engelsma et al., 2003) and in the channel catfish *Ictalurus punctatus* (Martins et al., 2011). This would indicate that temperature could have an effect on the hematology of fish modifying the kinetic of hematic cells (Martins et al., 2011; Engelsma et al., 2003)

Photoperiod is a key factor for maintaining the physiological balance of fish, since several organs participate in receiving external light signals (Li et al., 2016). Consequently, photoperiod manipulation is another common technique employed in fish aquaculture in order to optimize the production of a species (Boeuf and Le Baile, 1999; Bromage et al., 2001; Guerrero-Tortolero et al., 2010; Stuart and Drawbridge, 2011; Gunnarsson et al., 2012; Aragón-Flores et al., 2017). Effects of stress induced by photoperiod manipulation on hematology in captive fish have been assessed (Solomon and Okomoda, 2012a,b). However, few studies have been carried out with variable hematological responses (Srivastava and Sanjeev, 2010). In the African catfish haematological parameters PCV, MCHC, MCH, WBC, RBC, Hb, Hct, and PLT presented variations according to photoperiod exposed (Solomon and Okomoda, 2012b). Fish submitted in a photoperiod of 24 hours of light (24L:00D) presented the

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lowest value of WBC, Hb, Hct, RBC, and PCV compared to fish submitted to a photoperiod of 24 hour dark (24D:00L) or 12 hours light (12L:12D). According to Solomon and Okomoda (2012b), the low level of PCV, Hct and Hb appears to be linked to a reduction in RBC, which seems to be associated to a depletion of ATP (Emelike et al., 2008), inability to transport excess sodium out of the cell membrane and a consequent haemolysis (Guyton and Hall, 2005). Whereas, individuals of Indian catfish exposed to artificial photoperiod of 24L:00D and 00L:24D, the haematological parameters RCB and WBC did not presented differences in both artificial photoperiod regimes. Nevertheless, a differential leukocyte count showed a lymphopenia and neutrophilia in fish submitted to 24L:00D (Srivastava and Choudhary, 2010), which seems to be a characteristic of fish under stress as a direct cytolytic effect of cortisol on lymphocytes or as a distribution of immunological cells in lymphoid tissues (Espelid et al., 1996; Grzelak et al., 2017). In the great sturgeon *Huso huso*, changes in haematological parameters was observed due to the stress caused by photoperiod manipulation. An increment in Hct accompanied by a reduction in Hb and erythrocytes was found in fish under extreme 24L:00D and 00L:24D light regimes, what denotes the development of a possible anemia (Bani et al., 2009).

Salinity is also extensively studied because it is considered a determining growth and survival factor in fish farming (Lisboa et al., 2015; Baliarsingh et al., 2018). There is also a relationship between salinity stresses associated with hematological alterations, which can have a physiological impact on the immune system (Choi et al., 2013). In Nile tilapia, the haematological parameter Hct and Hb presented a decreasing tendency accompanied by a drop in RBC, probably as a consequence of changes in the water content in the blood due to exposure to an increasing hyperosmotic environments (Bosisio et al., 2017; Elarabany et al., 2017).

Meanwhile, WBC does not present significant differences in increasing salinity environments (Bosisio et al., 2017), but not so for environments with declining salinity, where a lymphopenia, neutrophilia and monocytosis can be observed leading to immune dysregulation (Choi et al., 2013). Whereas, the euryhaline species *M. cephalus* submitted to salinity of 25 and 45‰ reported also lowest levels of RBC, Hb, Hct compared to fish at salinity of 35‰, while WBC level was highest in fish at 25‰ and lower at 45‰ (Fazio et al., 2013c). The reduction in RCB, Hb, and Hct parameters may be attributed to salinity-induced osmoregulatory dysfunction (Girling et al. 2003), and decrement in WBC indicates an immunosuppressive effect some hemorrhagic injury caused by variation in salinity (Anyanwu et al., 2007). For the freshwater fish *Notopterus notopterus*, the haematological parameters Hb, Hct, RBC, WBC along with other blood index were raised after exposure to an increasing saline medium, this as response of fish trying to cope up with the changing salinity condition of the water (Kavya et al., 2016). Through induction of splenetic contractions and the subsequent mobilization of stored erythrocytes, in addition to an increase in muscle activity and the concomitant movement of water from plasma to muscle (Kavya et al., 2016). Then, understanding how seasonal variations can influence haemotological parameters can help to optimize husbandry practices (Faggio et al., 2014a).

The characteristics of farming system also seem to influence the haematological characteristics of cultured fish species. According to a study conduced by Fazio et al. (2013b) with the gilthead sea bream, the fish rearing with different aquaculture system have different baseline haematological value. Fish in onshore farming system tend to present lower value of RBC, Hct and WBC and higher value of MCV, MCH, MCHC, and Hb than fish in offshore farming system. The increase in MCV and Hb reported in onshore system could be due to compensatory mechanism to balance the low value of RBC; likewise the low value of WBC indicates a weakened defense in the fish due to a much lower present water quality than in an offshore system (Fazio et al., 2013a). However, fish in recirculating systems tend to present lower levels of hematological variables than fish in tidal systems probably to the relatively high physical and metabolic activity in fish, which are known to elicit a higher erythrocyte to plasma ratio in response to tidal shifts (Akinrotimi et al., 2010, 2011). Then, water quality in aquaculture is an important factor to consider since it directly influences the evaluation of haematological parameters as well as fish health (Kucuk, 2010; Fazio et al., 2013b; Gorjipour, 2014). Additionally, acclimation to captivity, a procedure commonly used in aquaculture, is a stressing factor able to alter the physiology of fish that in extreme cases results in mortality (Akinrotimi et al., 2007, 2009). Acclimation procedure (method and period) appears to exert an effect on fish haematology (Gabriel et al., 2004, 2011; Ezeri et al., 2004; Akinrotimi et al., 2007, 2010). In most of these studies a significant decline in the blood parameters RBC, Hct, and Hb is observed after a period of acclimation as reported for African catfish (Gabriel et al., 2011), *Sarotherodon melanotheron* (Akinrotimi et al., 2007), *Tilapia guineensis* (Akinrotimi et al., 2010), Nile Tilapia (Gabriel et al., 2011), and *M. cephalus* (Faggio et al., 2014b). This significant reduction in these parameters can be an indicator of severe anemia caused by acclimation stress effect (Akinrotimi et al., 2010; Faggio et al., 2014b). Notwithstanding, fish may be able to recover from an anemia caused by various adverse environmental and/or aquaculture factors as demonstrated in *M. cephalus* (Fazio et al., 2015). After a reduction in RBC, Hct, Hb, and WBC due to a bleeding period, fish prepare themselves for the persistence of the stress, reorganizing the haematopoietic response in the kidney to contrast the injury from anemia (Kondera, 2011; Fazio et al., 2015). On the other hand, an increase in

WBC is reported in most cases, which may be as a result of recruitment of more cells to combat the effect of acclimation in an attempt to maintain external homeostasis (Gabriel et al., 2011). Hence, acclimation-induced stress causes alterations in blood, which react in response to disturbances in both metabolic and haem activities of fish exposed to acclimation to captivity (Akinrotimi et al., 2010; Faggio et al., 2014b,c). Handling and transportation of fish are factors normally employed into aquaculture that may also lead to metabolic disturbance; enzymatic dysfunction, haematological variations, and several other malfunction in the fish (Kurovskaya and Osadchaya, 1993). According to a study carried out by Adeyemo et al. (2009) in the African catfish, handling and transportation stress can cause changes in haematological parameters Hct, Hb, and RBC, however, in a non-significant way, compared to a non-stressed fish group, but a significant decrease in WBC, which can make the fish susceptible to disease, parasite infection and even death (Wiik et al., 1989). Thus, changes in the composition of circulating WBC can be more reliable indicators of chronic crowding stress (Pickering and Pottinger, 1987) as reported also in the pejerrey *Odontesthes bonariensis* with a described lymphopenia and neutrophilia (Zebral et al., 2015). Presumably glicocorticoid hormones can modulate the lymphocytes redistribution from blood to another tissues, and stimulate the release of neutrophils from leucopoetic organs into the blood (Dhabhar et al., 1996; Espelid et al., 1996; Grzelak et al., 2017). On the other hand, the use of specific substances as immunostimulant is being introduced into fish farming routine procedures in order to improve the fish health with significant effect on haematological and biochemical parameters (Kumari and Sahoo, 2006; Yonar et al., 2012; Carbone and Faggio, 2016; Hoseinifar et al., 2018). In *C. carpio carpio*, propolis has a stimulating effect on the immune system, and a protective action on the haematogical parameters RBC, Ht, Hb

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counteracting the pesticide-induced toxicity such as chlorpyrifos (CPF) or Malathion (Yonar et al., 2012; Yonar et al., 2014). Both CPF and Malathion are broad-spectrum organophosphate pesticides for agriculture, domestic and public health purposes (Ali et al., 2009; Moore at al., 2011). Exposure to these pesticides can affect growth, swimming ability, and depletion of anti-oxidant system, biochemical and haematological parameters among other, even at a low concentration (Brewer et al., 2001; Girón-Pérez et al., 2006; Sweilum, 2006; Venkataramana et al., 2006; Huculeci et al., 2009; Tripathi and Shasmal, 2010; Yonar et al., 2014; Yonar et al., 2012; Yonar, 2018; Ural, 2013; Narra et al. 2015; Zahran et al., 2018). Another substance with immunostimulatory, anti-inflammatory, and anti-oxidant effects is the Gum Arabic (GA) (Cuesta et al., 2005). According to Faggio et al. (2015), fish of *M. cephalus* fed with 12% GA-pellets presented an increment only in TC values with no adverse effects on RBC, Hct, Hgb, WBC, MCV, MCH, MCHC values. Then, a positive effect on TC value suggests an immunostimulatory action by GA on fish (Passantino et al., 2005). Finally, hematology is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Kori-Siakpere et al., 2005; Fazio, 2019) by the variants commonly used in aquaculture (Valenzuela et al. 2007; Corrêa et al. 2017),

## 5. Mitochondria as a tool in haematological analyzes

In fish, erythrocytes have been demonstrated to possess complete cellular machinery with functional ribosomes (Lane and Tharp, 1980), and mitochondria (Ferguson and Boutilier, 1989; Pica et al., 2001; Moyes et al., 2002; Rey Vázquez and Guerrero, 2007), thus allowing protein synthesis and full cellular activity (Currie et al., 1999). The red blood cells are long-living cells with a relatively high level of

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respiratory activity. This determines the importance of the studies on the evaluation of the energy potential of mitochondria in the erythrocytes (Silkina et al., 2017). Moreover, due to their sensitivity to xenobiotics, fish erythrocytes are often used to evaluate xenobiotic-induced damage to different cellular compartment (Tiano et al., 2003; Witeska, 2013). Mitochondria are employed to investigate in great detail the mechanism of toxicity of xenobiotics, because of the key role of these organelles in the mechanism of cell death (Petit et al., 1995; Zamzami et al., 1995; Burgos Aceves et al., 2018a; b;). This may be due to mitochondria being both the source and the final target of free radicals effects. It seems that mitochondrial susceptibility to xenobiotics is associated to some factors. One of this is the presence of cytochrome P450s in mitochondria, which can activate chemicals that are relatively nonreactive prior to metabolism, such as PAHs and mycotoxins (Dong et al., 2009). On the other hand, the high lipid content of mitochondrial membranes facilitates accumulation of lipophilic compounds such as polycyclic aromatic hydrocarbons (PAHs) (Backer and Weinstein, 1982), some alkylating agents (Wunderlich et al., 1972), and certain organic chemicals, particularly amphiphilic xenobiotics such as ethidium bromide, paraquat, 1-methyl-4-phenylpyridinium (MPP+), and others (Cohen, 2010). Cationic metals, such as Pb, Cd, HG, and Mn, have also been shown to accumulate in mitochondria preferentially (Atchison and Hare, 1994; Bucio et al., 1999; Gavin et al., 1992; Sokolova et al., 2005; Gomes et al., 2015). A study done by Tiano et al. (2003) evidenced the toxic effect of tributyltin chloride (TBTC) on erythrocytes and leukocytes in fish of rainbow trout. The TBTC is able to display a consistent drop in mitochondrial membrane potential both in erythrocytes and leukocytes, and release of proapoptotic proteins (cytochrome c, caspase-3), and consequently mitochondrial pathways are able to trigger apoptosis in these cell type. Effects that can be reversed through an increase in antioxidant and detoxifying enzyme activities by supply diets with low-fat content (Lionetti et al., 2012).

Finally, mitochondria are essential organelles for ATP production, cell life-and-death process (Lee and Wei, 2012), and primary source of reactive oxygen species (ROS), which can be both cytotoxic and regulatory (Dröge, 2002). Then, the ability of cells to produce heat due to ATP heat-producing hydrolysis for a long period of time indicates the importance of the study of the mitochondrial bioenergetic complex in erythrocytes (Silkina et al., 2017). Then, mitochondria seem to be a convenient material to study the bioenergetics activities, biogenesis and disappearance of mitochondria in these cells as well as in red blood cells in general.

## 6. Perspectives

Unfortunately, the accumulation of various types of xenobiotics in water bodies is increasing as the amount and types of wastewater generated by a snowballing human activity. Therefore, it is necessary to carry out more basic studies for all new pollutants on the fish haematology that allows having a starting point before any environmental alteration. It is also necessary to consider that wild fish from natural environments may exhibit different physiological behaviors related to their survival strategies. In addition, another important point to consider when making an environmental or aquaculture assessment is to take into account additional sources such as dietary exposure (Putti et al., 2015; Meador et al., 2017; Lepretti et al., 2018). A more multidisciplinary framework in field studies is also essential for better understanding wildlife disease outbreaks and multi-trophic impacts on ecosystems. Therefore, a complementary bioenergetics and dynamic mitochondrial study would give greater strength to a hematological analysis both in environmental and aquaculture evaluations. Since there is no standardized method to determine the health of the fish or the environment, it is the combination of indicators of impairment that will give us the best diagnostic picture (Todgham and Stillman, 2013). Haematology is still an opaque science for wildlife but promoting its standardization of pre-analytical procedures plus some suggestions for a more systematic examination of blood smears to increase the diagnostic value of blood data. Establishing various hematologic changes that occur in fish is crucial in assessing their health and also provides the opportunity to expand the use of some fish as models for human disease.

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## Declaration of interest

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