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Fish haematology: A review

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Abstract
Fish Red-Blood Cells (RBCs) are nucleated cells that can modify the expression of several genes in response to stimuli, contributing to the immune system's equilibrium. The erythrocyte of a fish can be thought of as a prototype for the red cells. It's an oval, flattened, biconvex disc that's perpetually nucleated and haemoglobin-laden. Erythropoiesis begins in the yolk sac and is followed by the intermediate cell mass in many fishes. These are the origins of the transient, primitive generation red cells, which initially emerge in fishes and are later found in all vertebrate classes, including mammals. In cyclostomes, dipnoi, and chondrichthyes, production of definitive generation erythrocytes is localised in evolutionary "pre-splenic" tissue of the gastrointestinal tract or in the spleen, whereas in teleosts, it is frequently located in the kidneys with or without splenic participation.

Keywords: Fish haematology, fish red-blood cells, erythropoiesis

Introduction
Fish erythrocytes are nucleated ellipsoidal cells with a range of sizes (102–800 fl) and lifespans (13–500 days). Erythrocyte count is highly seasonal and is influenced by fish activity, water temperature, and dissolved oxygen content, as well as other environmental conditions. It is also influenced by factors such as age, sex, diet, and reproductive status, and it can vary amongst populations of the same species. In less active species, it varies from 0.5–1.5 10^6 /mm^3 to 3.0–4.2 10^6 /mm^3 in more active ones. Erythrocytes are absent in Antarctic ice fishes that have evolved to cold, well-oxygenated water. In teleost fishes, the head kidney is the primary erythropoietic location, and the erythropoiesis process is comparable to that of other vertebrates and involves the same precursors. Fish have a porous barrier between hematopoietic tissue and circulating blood, with many immature cells accounting for more than 10% of all erythrocytes. Fish erythrocytes, like those of other vertebrates, include tetrameric hemoglobins with varied oxygen affinity, which are lower in species that live in well-oxygenated water than in those that live in hypoxia. Blood often contains many haemoglobin isoforms with varied oxygen affinity, which is thought to be an adaptation to the fluctuating oxygen concentration in water. Because fish erythrocytes are vulnerable to pollution, their morphological evaluation could be employed as a toxicity bioindicator.

Erythrocytes
In fish, erythrocytes are the most abundant blood cells 98–99 percent (Fange 1994) [2]. Fish erythrocytes are ellipsoidal and include a nucleus, just like those of other vertebrates with the exception of mammals. Their dimensions vary significantly: long diameter 8.8–17.1 m and short diameter 6.9–12.9 m (http://www.genomesize.com/cellsize/fish.htm). In teleost fishes, Lay and Baldwin (1999) discovered an inverse association between erythrocyte size and aerobic swimming capacity. They explain that in smaller cells, a larger surface area to volume ratio leads to a shorter diffusion distance and faster oxygen transport. Each species has a different amount of erythrocytes (RBC). According to Soldatov (2005), some species have a very low erythrocyte count (0.5–1.5 10^6 /mm^3) while others have a very high RBC (3.0–4.2 10^6 /mm^3), and the discrepancies are primarily due to locomotor activity. The number of erythrocytes in fish is highly influenced by the environment (mainly by temperature and dissolved oxygen level). As a result, seasonal variations in erythrocyte counts occur, making it difficult to establish hematologic reference values (Luskova 1997) [10]. In healthy *Cyprinus carpio*, erythrocyte counts can range from 0.79 to 2.90 10^6 /mm^3 (Witeska 2003) [22], while in *Oncorhynchus mykiss*, they can range from 0.28 to 1.34 10^6 /mm^3 (Rehulka *et al*., 2004) [15].

Erythropoiesis

The erythropoietic organ of teleosts is the head kidney, which has a cellular composition similar to mammalian bone marrow, whereas the spleen has a weak erythropoietic activity (Fange 1994) [2] and the head kidney serves mostly as a blood reserve, providing erythrocytes to the circulation when O2 demand is high (Valenzuela *et al*. 2005) [18]. Hematopoietic stem cell, myeloid stem cell, CFU-E, pro erythroblast, basophilic erythroblast, polychromatic erythroblast, and acidophilic erythroblast are the precursors of fish erythropoiesis, according to Fijan (2002a, 2002b) [3-4]. The cells of the erythroid line can be distinguished by morphological characteristics starting with pro erythroblast. Because the blood in fishes often contains a high proportion of immature erythrocytes, mostly polychromatic and acidophilic erythroblasts that undergo their final maturation within the circulation, the barrier between circulating blood and the hematopoietic system, which is well developed in mammals, appears to be much weaker in fish (Fange 1994) [2]. Fish erythrocytes can undergo amitotic divisions under hypoxic environments (Soldatov 1996) [17]. Young mature erythrocytes of fish may still produce haemoglobin, according to Schindler and de Vries (1986) [16].

Haemoglobin

Tetrameric haemoglobin is found in fish erythrocytes, just as it is in other vertebrates. Teleost fish hemoglobins have a wide range of oxygen affinities. Hypoxia tolerant fish species (such as tench, pike, and carp) have a high hemoglobin-oxygen affinity, whereas oxygen-rich fish species (such as salmonids) have hemoglobins with a lower oxygen affinity (Nikinmaa 2001) [12]. According to Nikinmaa (2001) [12], one of the conceivable mechanisms for fish to adjust to varying dissolved oxygen levels is a shift in the proportion of distinct hemoglobins with differing oxygen affinity. Other variables that increase the oxygen affinity of fish hemoglobins include erythrocyte adrenergic swelling (Lecklin *et al*., 2000) [9]. Because the affinity of haemoglobin for oxygen rises as the MCHC decreases, this trait is clearly an adaaptive response to increased oxygen demand (Nikinmaa 2001) [12]. The action of protons – the Bohr effect– and a similar effect of CO_2 are the key factors lowering affinity. Under conditions of elevated metabolic demand, such as metabolic acidosis, the Bohr effect enhances oxygen release at the tissue level (De Souza and Bonilla-Rodriguez 2007) [1]. Another effect of low pH on haemoglobin oxygen affinity - the Root effect – describes the fact that haemoglobin cannot be fully saturated with oxygen in some teleost fishes (Kunzmann 1991). The Root effect, according to De Souza and Bonilla-Rodriguez (2007) [1], is required to fill the swim bladder and give oxygen to the retina, which lacks capillaries.

Hematocrit

The relationship between the hematocrit and blood viscosity is a significant consideration for fish physiologists (Wells and Weber, 1991) [20]. Large, energetic fish have a high muscular oxygen demand, which might cause erythropoiesis stimulation in the head kidney (Jawad *et al*., 2004).As a result, the physiological hematocrit of fast-swimming pelagic fish and fish that live sedentarily or in benthic habits vary (Wells and Davie, 1985) [19]. The hematocrit can be increased by anaesthesia (Phuong *et al*., 2017) [14] while malnutrition, sickness, or exposure to environmental contaminants can lower hematocrit and haemoglobin levels in fish (Witeska 2015) [21]. Anemia, a unique pathologic stress reaction, is defined by non-physiologically low hematocrit readings. Anemia in fish can be quickly recognised by looking at the gills, but more in-depth blood tests can assist determine the source of anaemia (Noga, 2010). The electrolyte–water balance of the fish blood can influence the hematocrit and Hb values related with erythrocyte count (Islam *et al*., 2020).Stressed freshwater fish have lower plasma sodium levels, which triggers counter-transporting ion channels on erythrocyte membranes (Martemyanov, 2013). The increased ion concentration causes an input of water, which causes the erythrocytes to enlarge and increase their oxygen binding ability. To compensate for the increasing oxygen demands, these responses are accompanied by the release of extra erythrocytes from splenic reserves.

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