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Characterization of Blood Cells and Hematological Parameters of Yellowfin Sea Bream (*Acanthopagrus latus*) in Some Creeks of Persian Gulf

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Abstract: In this study we detect different levels of blood cells and hematological parameters in five creeks of Mahshahr region in North West of Persian Gulf with different levels of pollutant to characterized hematological response of Yellowfin seabream to environmental pollutant and undesirable materials. Numbers of Blood leukocytes was performed by a hemocytometer Neubauer under the light microscope. The leukocyte differential count was made in peripheral blood smears, giving the neutrophils value of differential neutrophils and the mononuclear value of differential lymphocytes plus monocyte and eosinophile. Hematocrit values were determined in a microhematocrit centrifuge. Hemoglobin levels were determined colorimetrically by measuring the formation of cyanomethemoglobin. Result declared significance increase of differential monocyte and neutrophil within higher considerable values than those of the clean station, beside significance decrease of Hb, Ht, leukocyte count, differential lymphocyte, eosinophyle and MCHC ($P>0.05$) with lower considerable values than those of the clean station and neutrophilia, monocytosis, lymphopenia and eosinopenia were find in the creeks. Results of the present investigation showed that pollutants have direct correlation with fish blood abnormalities and also indicated that the different in environmental condition of marine ecosystems may cause several changes in the hematological parameters of the studied fish.

Key words: Blood · Hematology · Persian Gulf · Yellowfin Sea Bream

INTRODUCTION

Blood is the most accessible component of the vertebrate body fluid system and has frequently been examined to assess physiological status [1]. Although not self-evident, blood is classified as a connective tissue. All blood cell components (i.e. erythrocytes, leukocytes and thrombocytes) have their origin in stem cells found in bone marrow. Poietins, or stimulating factors, regulate the fate of a stem cell, whether it becomes an erythrocyte (erythropoietin), leukocyte, or thrombocyte.

The hematological variables include the percentage of blood volume consisting of red cells (hematocrit, or Hct), red blood cell count per unit blood volume and hemoglobin (Hb) concentration. These are the primary indices (directly measured) and a series of secondary indices may be calculated, including mean red cell volume and mean erythrocyte hemoglobin [2]. However, Houston [1] pointed out that these indices were originally derived for human and veterinary health studies and that error can be introduced into data on fish blood because fish red blood cells have a different shape, membrane flexibility and erythron profile compared to those in mammalian blood. Proper oxygen transport, immune function and clot formation result when normal numbers of erythrocytes, leukocytes and thrombocytes are present, respectively [3].

The hematology tests routinely performed during toxicology studies evaluate erythrocytes, leukocytes, platelets and coagulation. Many automated cell counters can determine red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count and platelet count. Because erythrocytes of the common laboratory species are smaller than human erythrocytes, the cell counters must be adjusted to ensure accuracy. Cell counters can now perform WBC differential

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counts on animal blood, but microscopic examination of a stained blood film is often necessary to confirm results when abnormalities occur [4].

Creeks and estuaries ecosystems are subjected to discharge from anthropogenic activities and their living aquatic animals are exposed to severe damage due to increased industrialization and urbanization near the coastal areas. Mahshahr creeks, located in the north west of Persian Gulf, faces this problem because it is being surrounded by polluting industries, which deposit pollutants directly into the creeks.

To date, little is known about the hematology of yellowfin sea bream, which is one of the most important species in mariculture worldwide, so we have revealed an interesting pattern of response on the hematological variables in stressed fish.

Blood characteristics are very sensitive to environmental pollutant and their response to undesirable materials is very fast, so in this study we detected different levels of blood cells and hematological parameters in five creeks of Khoor-Mousa in Mahshar region with different levels of pollutant to Characterized hematological response of Yellowfin Sea Bream to environmental pollutant and undesirable materials.

MATERIALS AND METHODS

Environment Design: According to our past data, from 26 creeks in Mahshahr region (northwest of Persian Gulf) we choose four more pollutant creeks (Jafari, Ghazaleh, Majidieh and Petroshimi) and one less pollutant as control treatment (Zangi). For every creek we choose three station and for every station two yellowfin sea bream with the same size (170 g) and same sexually (all immature male) were caught with hooks and transferred immediately in a fiberglass tank equipped with specific creek water and chargeable aeration (SOBO, China) to the Mariculture Research Station of the South Iranian Aquaculture Research Center, Mahshahr, Iran.

Blood Analyses: To obtain blood samples, fish were quickly taken out from the water and held firmly on a bench with a cloth covering the head and blood was withdrawn from caudal vessels by a vacuum heparinized syringes and needle containing 0.1mL of anticoagulant (after filling up and expelling about 1.0 mL), to the Eppendorf tubes, whole blood withdrawal process took less than 1 min per fish. Handling time was less than 1min to minimize stress effects.

Hematology Analysis: Determinations of the number of CBC tests were performed immediately on fresh blood.

Numbers of Blood leukocytes was performed by diluting heparinized blood with Giemsa stain at 1:30 dilution and cells were counted using a hemocytometer Neubauer under the light microscope [5].

The leukocyte differential count was made in peripheral blood smears stained by Merck Giemsa [6], giving the Neutrophils value of differential neutrophils (100 leukocytes count) and the Mononuclear value of differential lymphocytes plus monocyte and eosinophile (100 leukocytes count).

Giemsa staining enabled the specific identification of lymphocytes from other types of leukocytes. Lymphocyte numbers were determined by direct counting under the microscope using a Neubauer chamber. By means of a suspension based in methylene blue, circulating lymphocytes of sea bream can be distinguished from erythrocytes and thrombocytes have morphological features which differentiate the two cell types. Criteria for identification of lymphocytes were as given in Hibiya [7].

Hematocrit values (Ht %) were immediately determined after sampling by placing fresh blood in glass capillary tubes and centrifuged for 5 min at 10,500 rpm in a microhematocrit centrifuge (Hettich, Germany) then measuring the packed cell volume [8], Hematocrit readings were performed with the aid of a microhematocrit reader.

Hemoglobin levels (Hb mg/l) were determined colorimetrically by measuring the formation of cyanomethemoglobin according to [9].

Mean corpuscular hemoglobin concentration (MCHC) were calculated from RBC, Ht and Hb according to Lee *et al.* [9] as $MCHC(mg\ l^{-1}) = Hb(mgdl^{-1}) / Ht(ratio)$.

Statistical Analyses: One-way analysis of variance ANOVA with Duncan Post Hoc was used to determine significant differences. The differences between means were analyzed at the 5% probability level (p value of less than 0.05 was considered as statistically significant). Data are reported as means ± standard deviation ($\bar{x} \pm SD$). The software SPSS, version 11.5 (SPSS, Richmond, Virginia, USA) was used.

RESULTS

Five sampling stations (Zangi, Jafari, Ghazaleh, Majidieh and Petroshimi respectively) from Mahshahr coastal waters which were considered important for their commercial and recreational potential were selected to

enumerate the presence of mercury and its synergetic action on the liver of A. latus. Station Zangi had lower levels of mercury contamination in all measurements and choose as clean station. Other Stations had higher levels of mercury contamination in all measurements and were choose as infected station. Stations Majidieh and Petroshimi were noticeably close to an area of industrial activities (oil and petrochemistry respectively) and higher amount were predictable.

Results of *in vivo* hematological and immunological analysis are presented in table 1. All in indices exhibited high significant analysis of variance ($P<0.05$), but the statistical analysis did not reveal any significant difference ($p>0.05$) between control groups and differential eosinophils. *In vivo* result declared significance increase of differential monocyte and neutrophil within higher considerable values than those of the control group, beside significance decrease of Hb, Ht, leukocyte count, differential lymphocyte, eosinophyle and MCHC ($P>0.05$) with lower considerable values than those of the control group.

The correlation between mercury with hematological parameters was statistically tested by analyzing the data obtained during the five sampling creeks for in vivo indices. Correlation coefficients between water mercury concentrations and hematological and immunological indices were significant in Hb, Ht, Monocyte, Neutrophil and Eosinophils ($P<0.05$), that among significant parameters Monocyte, Neutrophil, Eosinophils were positive and Hb, Ht correlation were negative in correlate with water mercury and among insignificant indices only Monocyte had positive and other indices had negative correlation (Table 2).

Result of sediment correlation show only Leukocyte had significant negative correlate whereas within insignificant parameter Hb, MCHC, Monocyte and Eosinophils had positive correlation with sediment mercury and Ht, Lymphocyte and Neutrophil had negative correlation (Table 3).

In order to evaluate the response of A. latus to mercury concentrations, a linear model and a sigmoid model were tested and compared. The variation of

Table 1: In vivo hematological activities of yellowfin seabream exposed to water mercury.

	Zangi	Jafari	Ghazaleh	Majidieh	Petroshimi
Hb (mg/l)	8.98 ± 0.3^{b}	9.56 ± 0.1^{a}	7.66 ± 0.3^{c}	7.8 ± 0.6^{c}	8.68 ± 0.3^{b}
Ht (%)	26.1 ± 1.9^{bc}	30.1 ± 1.7^{a}	24.1 ± 1.1^{cd}	23.1 ± 2^{d}	26.6 ± 1.5^{b}
MCHC(mg/l)	0.34 ± 0.02^{a}	0.31 ± 0.01^{b}	0.31 ± 0.01^{b}	0.33 ± 0.007^{ab}	0.32 ± 0.01^{ab}
Leukocyte (/ml)	12141 ± 1313^{a}	10821 ± 526^{b}	11385 ± 262^{ab}	11381 ± 952^{ab}	10563 ± 1161^{b}
Lymphocyte (%)	78.3 ± 2^{a}	71 ± 4^{b}	71.8 ± 3^{b}	74.6 ± 2^{ab}	72.6 ± 2^{b}
Monocyte (%)	1.83 ± 0.75^{b}	3.16 ± 0.75^{a}	2.83 ± 0.75^{ab}	3.33 ± 1.63^{a}	3.66 ± 0.81^{a}
Neutrophil (%)	16.8 ± 0.7^{b}	20.3 ± 2.1^{a}	22 ± 3.2^{a}	19.3 ± 2.5^{ab}	19.6 ± 1.5^{a}
Eosinophils (%)	4.5 ± 1.5^{a}	4.5 ± 1.5^{a}	3.1 ± 1.1^{a}	2.6 ± 1.6^{a}	3.1 ± 1.1^{a}

Table 2: In vivo correlation of hematological activities of yellowfin seabream with water mercury.

	Hb	Ht	MCHC	Leukocyte	Lymphocyte	Monocyte	Neutrophil	Eosinophils
(r)	-0.70^{**}	-0.49^{**}	-0.25	-0.19	-0.35	0.43^{*}	0.46^{**}	0.49^{**}
(p)	0.00	0.00	0.18	0.30	0.05	0.01	0.01	0.00

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Table 3: In vivo correlation of hematological activities of yellowfin seabream with sediment mercury.

	Hb	Ht	MCHC	Leukocyte	Lymphocyte	Monocyte	Neutrophil	Eosinophils
(r)	0.01	-0.06	0.14	-0.36^{*}	-0.16	0.23	-0.02	0.08
(p)	0.93	0.74	0.45	0.04	0.37	0.21	0.88	0.67

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Table 4: In vivo curve fit linear regression of hematological activities of yellowfin seabream with water mercury.

	Hb	Ht	MCHC	Leukocyte	Lymphocyte	Monocyte	Neutrophil	Eosinophils
(r^2)	0.49	0.24	0.06	0.03	0.12	0.18	0.21	0.24
F	27.3	9.04	1.89	1.08	4.01	6.4	7.63	9.21
(p)	0.00^{**}	0.00^{**}	0.17	0.30	0.05^{*}	0.01^{*}	0.01^{*}	0.00^{**}

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level.

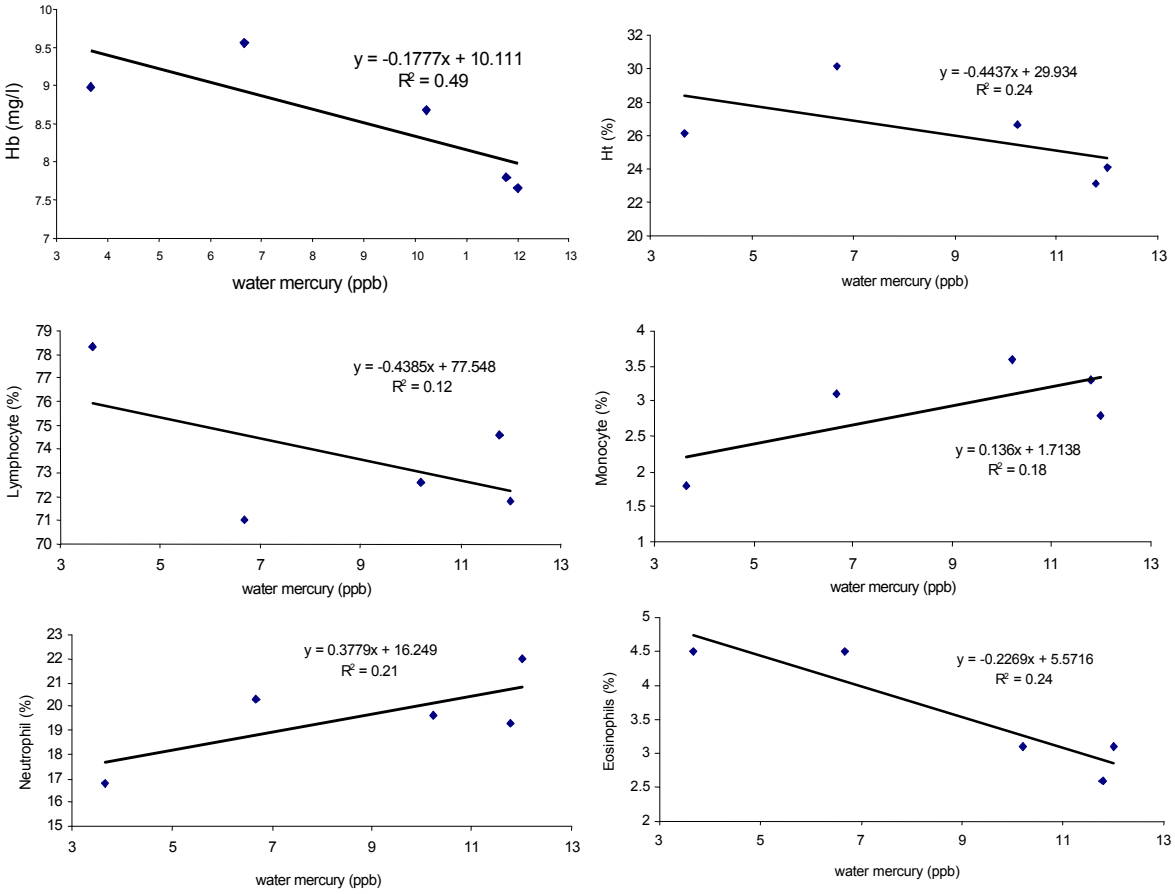

Fig. 1: Regressions model (Y = a ± bX) of Hb, Ht, Lymphocyte, Neutrophil and Eosinophils of the yellowfin seabream during in vivo exposed to different concentration of water mercury.

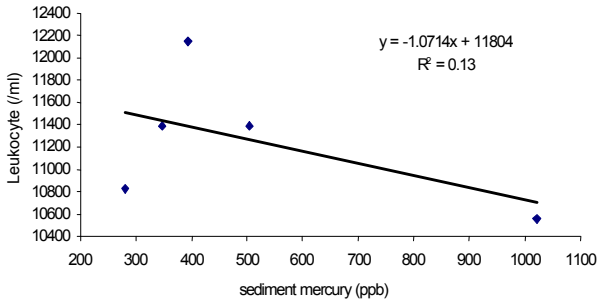

Fig. 2: Regressions model (Y = a ± bX) of Leukosyte of the yellowfin seabream during in vivo exposed to different concentration of sediment mercury.

Table 5: In vivo curve fit linear regression of hematological activities of yellowfin seabream with sediment mercury.

	Hb	Ht	MCHC	Leukocyte	Lymphocyte	Monocyte	Neutrophil	Eosinophils
(r^2)	0.00	0.00	0.02	0.13	0.02	0.05	0.00	0.00
F	0.00	0.11	0.57	4.27	0.80	1.59	0.02	0.18
(p)	0.93	0.74	0.45	0.04*	0.37	0.21	0.88	0.67

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level.

mercury concentration in the *in vivo* and *in vitro* condition were best fitted by a linear model equation, so linear regression model was found to fit well the relations between the concentrations of mercury with hematological parameters. Curve estimation regressions data were used to determine the relationship between water mercury and hematological content in different creeks. Except MCHC and Leukocyte, other parameters show significant linear regression ($P<0.05$) with water mercury (Table 4). Regressions model $Y = a \pm bX$ of significant indices are in fig 1.

Curve estimation regressions data were used to determine the relationship between sediment mercury and hematological and immunological content in different creeks. Except Leukocyte, other parameters did not show significant linear regression ($P<0.05$) with sediment (Table 5). Regressions model $Y = a \pm bX$ of Leukocyte is in fig 2.

DISCUSSION

Hematotoxins change quantitative and qualitative characteristics of blood cells to produce toxic symptoms. Hematotoxicity happen when some of these different blood components are present or structural anomalies occurring in blood components interfere with normal functioning [3]. Qualitative changes in blood cell components can result in pollution. Microcytic hypochromic anemia results when erythrocytes have low hemoglobin content. Although adequate in quantity, the quality of these "small, less colored" red blood cells prevents them from carrying the normal amount of oxygen [3].

Five tests are commonly used to measure the quantitative and qualitative aspects of blood. Three of these, the red cell count, white cell count and platelet count, are quantitative measurements of blood components. The other two, hemoglobin (Hb) and hematocrit (HCT), are indicators of the oxygen-carrying capacity of blood. Collectively, these tests, plus others, are termed a complete blood count (CBC) with differential (DIFF). The CBC/DIFF provides information on the number, variety, percentages, concentrations and quality of blood components [3].

The most common hematology findings in toxicology studies are mild decreases in RBC count, hemoglobin concentration and hematocrit (the percentage of whole blood made up of erythrocytes) that is like our finding in current research. A decrease in hemoglobin and hematocrit levels can indicate an anemic condition [10]. Clomazone induced a reduction in the hematocrit value of silver catfish after 96 and 192 h, indicating a possible anemic state. However, this condition was easily overcome after the recovery period in clean water [11]. Also decreases in both values oh Hb and Ht after exposure to Cadmium were found [12].

The examination of leukocytes is includes the quantitative determination of total and differential WBC counts and the qualitative assessment of cellular morphological abnormalities. The differential WBC count enumerates granulocytes (neutrophils, eosinophils and basophils), lymphocytes and monocytes [4]. Increased numbers of these cells are called neutrophilia, eosinophilia, basophilia, lymphocytosis and monocytosis, respectively. Neutropenia, eosinopenia and lymphopenia refer to decreases, so in current study neutrophilia, monocytosis, lymphopenia and eosinopenia were find in creeks. However the normal cell counts for basophils and monocytes are so low that decreases are difficult to recognize.

Relative counts (percentages) for the different types of leukocytes, obtained by doing the differential count, are of little or no value without knowledge of the total WBC count.

Differential WBC counts should always be reported as absolute numbers. Neutrophils and lymphocytes are the principle cell types found in peripheral blood and toxicologic effects on leukocytes usually involve one or both of these cell lines. Although primary effects occur, the changes observed are most commonly secondary changes in response to primary toxicity of other tissues or organ systems [4].

A steroid or stress-induced leukocyte response refers to a combination of changes observed in fish receiving corticosteroids or producing increased endogenous corticosteroids because of some stressful condition. It generally consists of a mature neutrophilia, lymphopenia and sometimes monocytosis depending on the fish species. The mature neutrophilia develops as a consequence of increased release of segmented cells from the bone marrow storage pool, decreased margination of cells, decreased movement of cells into tissues and increased stability of lysosomal membranes. Lymphopenia results from steroid-induced lysis and cell redistribution. Eosinopenia develops as a result of decreased production and release from the bone marrow. Monocytosis, when it occurs, is thought to result from mobilization of marginated cells. It is interesting that the stress-induced leukocyte response is a relatively infrequent finding in toxicology studies even though the study design or the test material often creates physical conditions that appear to be quite stressful [4].

The primary function of the neutrophil is phagocytosis of small particulate matter (e.g. bacteria). The neutrophil is also an integral cellular component of inflammation. It is therefore not unusual to observe neutrophilia secondary to nearly any inflammatory lesion caused by a test material.

Unless moderately severe, however, dermal inflammation might not induce increases in neutrophil count. The term *left shift* indicates an increased number of immature neutrophils in circulation. A left shift can occur whenever an inflammatory lesion has a significant demand for neutrophils and immature cells are released from the bone marrow. Lesions that cause a left shift are almost always easily identified, if not by physical examination and the evaluation of other laboratory data, then certainly at necropsy. They frequently involve infectious organisms that have invaded tissue damaged by the test material [4].

These cells are responsible for a wide variety of immune system functions. Although there are many lymphocyte subpopulations, it is not possible to distinguish them by light microscopic examination. Lymphocytes are unique among leukocytes in that they recirculate; that is, lymphocytes leave the vascular system through venules in lymph nodes and ultimately return to the blood through the thoracic duct. They are long-lived cells compared with other leukocytes.

Lymphopenia occurs most frequently as a part of the steroid or stress-induced leukocyte response. Agents that cause neutropenia, such as chemotherapeutic agents, will usually cause lymphopenia as well. Because of the many subpopulations of lymphocytes, it is difficult to know the biological significance of a small change in lymphocyte number [4].

Absolute eosinophil and monocyte counts are normally very low and quite variable. It is very unusual, therefore, to be able to detect toxicologic effects on these cell types.

The primary function of the monocyte is phagocytosis and digestion of large particulate matter such as senescent cells, necrotic cellular debris and large micro-organisms. Monocytes process antigens and present them to lymphocytes in a more antigenic form. Monocytosis can occur secondary to lesions involving extensive tissue destruction such as neoplasms with associated necrosis or hemolytic anemia [4].

Monocytes are circulating cells with a distinctly different lineage from that of granulocytes. Like granulocytes, they are phagocytic and migrate out of the circulating peripheral blood leukocyte compartment and into tissues where they differentiate into tissue macrophages. With proper stimulation, blood monocytes can have enhanced activities, but not to the degree of tissue macrophages. In response to inflammation, tissue macrophages secrete cytokines, various enzymes, reactive oxygen intermediates, antimicrobial peptides and arachidonate metabolites (prostaglandins and leukotrienes).

The key role of macrophages is to bridge non-specific with specific responses and they do this by acting as professional antigen-presenting cells [13].

Reite [14] has noted that eosinophilic granular cells are also abundant in salmonids, particularly in the intestine. Like mammalian mast cells, these cells readily degranulate upon stimulation with 40:80 poly-L-lysine and substance P. Phagocytosis also initiates degranulation.

In conclusion, results of the present investigation showed that pollutant have direct correlation with fish blood abnormalities and also indicated that the different in environmental condition of marine ecosystems may cause several changes in the hematological parameters of the studied fish.

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